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The evolving phenotype and long-term outcomes of Silver-Russell syndrome and the effects of childhood growth hormone treatment

Volume 1 of 1

by

Oluwakemi Lokulo-Sodipe

ORCID ID 0000-0002-8169-3384

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Abstract

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Human Development and Health

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Silver-Russell syndrome (SRS) is a disorder which causes pre- and postnatal growth failure. Maternal uniparental disomy for chromosome 7 and loss of methylation of the intergenic H19/IGF2 differentially methylated region at chromosome 11p15 are reported in 50-60% of cases. Birth weight is known to be inversely associated with adult cardiovascular disease and metabolic abnormalities, therefore low birth weight in patients with SRS may be associated with increased risk of cardiovascular and metabolic disease. However, adults with SRS are not routinely followed up and only recently has specific guidance on potential complications and a consensus on the management been published.

Growth hormone treatment in children born small for gestational age is known to increase muscle mass and reduce fat mass during treatment as well as increasing adult height. Specifically in SRS, growth hormone treatment has been shown to increase height velocity and the limited data available has suggested increased adult height. Furthermore, there is a paucity of evidence on body composition in SRS, quality of life in individuals with SRS, and whether or not growth hormone treatment affects these.

This thesis presents the work undertaken in a study of the adult phenotype of SRS. The objectives were to: recruit individuals aged ≥13 years with molecularly confirmed SRS; assess body composition, metabolic health and risk factors for cardiovascular disease; evaluate their quality of life, and compare those treated with growth hormone in childhood with those who did not receive treatment.

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Research Thesis: Declaration of Authorship

Print name:	Oluwakemi Lokulo-Sodipe

Title of thesis:	The evolving phenotype and long-term outcomes of Silver-Russell syndrome and the effects of childhood growth hormone treatment

I declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University;
2. Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
3. Where I have consulted the published work of others, this is always clearly attributed;
4. Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
5. I have acknowledged all main sources of help;
6. Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
7. Parts of this work have been published as:

Ballard LM, Jenkinson E, Byrne CD, Child JC, Davies JH, Inskip H, Lokulo-Sodipe O, Mackay DJG, Wakeling EL, Temple IK, Fenwick A. The lived experience of Silver-Russell syndrome: implications for management during childhood and into adulthood. Arch Dis Child 2018. doi: 10.1136/archdischild-2018-314952. [Epub ahead of print].

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Definitions and Abbreviations

bp	Base pairs
BIA	Bio-electrical impedance analysis
BMD	Bone mineral density
BMAD	Bone mineral apparent density
BMI	Body mass index
CGF	The Child Growth Foundation
DMR	Differentially methylated region
DNA	Deoxyribonucleic acid
DXA	Dual energy x-ray absorptiometry
e.g.	exempli gratia [for the sake of example]
GH	Growth hormone
GOM	Gain of methylation
HRpQCT	High resolution peripheral quantitative computed tomography
HRQoL	Health-related quality of life
ICR	Imprinting control region
ID	Imprinting disorder
IDFOW	The 'Imprinting disorders: finding out why' study
i.e.	id est [that is]
IGF	Insulin-like growth factor
IQ	Intelligence quotient
IU	International unit
IUGR	Intra-uterine growth retardation
FFM	Fat-free mass

FFMI	Fat-free mass index
FMI	Fat mass index
LOM	Loss of methylation
LBM	Lean body mass
LMI	Lean mass index
Mb	Mega base(s)
MS-MLPA	Methylation-specific multiplex ligand dependent probe analysis
MS-PCR	Methylation-specific polymerase chain reaction
MRKH	Mayer-Rokitansky-Küster-Hauser syndrome
NIHR	National Institute for Health Research
OFC	Occipito-frontal circumference
PCR	Polymerase chain reaction
RNA	Ribonucleic acid
RSS	Russell-Silver syndrome
SD	Standard deviation
SDS	Standard deviation score
SEIQoL-DW	Schedule for the Evaluation of Individual Quality of Life – Disability Weighted
SGA	Small for gestational age
SRS	Silver-Russell syndrome
UPD	Uniparental disomy
UK	United Kingdom
USA	United States of America
WRGL	Wessex Regional Genetics Laboratory
WHO	World Health Organization

Chapter 1 Background

1.1 Introduction

In 1953, Henry K. Silver and colleagues first described two patients with low birth weight, short stature, with 'hemihypertrophy' in one case and 'asymmetry' in the other. Café-au-lait macules were also described (1). The following year Alexander Russell presented five cases with low birth weight, low birth length, characteristic facies, short upper limbs, absent carrying angle or cubitus varus, incomplete supination, incurved fifth finger, a narrow chest, and general leanness (2). The facial features described were: triangular shape, small face relative to skull size, prominent nose, and a 'shark-like mouth'. It was later suggested that both authors had described different clinical features of the same condition, which was referred to as 'Russell-Silver type' dwarfism (3). The syndrome has subsequently been termed: 'Silver's syndrome' (4), 'Russell-Silver syndrome' (5-9), 'Silver-Russell dwarf' (10), 'Silver-Russell type of dwarfism' (11) and 'Silver-Russell syndrome' (12-14). The terms 'dwarf' and 'dwarfism' are no longer used and both 'Silver-Russell syndrome' and 'Russell-Silver syndrome' are accepted terms. There has been no absolute decision on terminology however the recent international consensus on the diagnosis and management used the term 'Silver-Russell syndrome' (15). For the remainder of this work 'Silver-Russell syndrome' and the abbreviation 'SRS' will be used unless original work is quoted or reproduced (with permission).

The clinical features of SRS include prenatal and postnatal poor growth with variable dysmorphic features. The incidence is estimated to lie between 1 in 50 000 and 1 in 100 000 (16). A study in Estonia demonstrated an incidence of 1 in 70 000 for molecularly confirmed cases therefore the true incidence may be greater than this calculation (17).

1.2 The clinical characteristics of SRS

1.2.1 Growth patterns in SRS

1.2.1.1 Pregnancy, intra-uterine growth and birth weight

In the early case reports, growth was reduced in one pregnancy but may have been affected by a road traffic accident in the seventh month of gestation (1) and threatened miscarriage was reported in three of five cases (2) and in 2/29 cases (14). 'Intra-uterine growth failure' was

proposed in the original description of SRS (2) and subsequently intra-uterine growth retardation (IUGR) has been widely accepted as a key feature of SRS in the absence of pregnancy complications (18).

True IUGR refers to the decline of the growth rate in utero and is distinct to a birth weight being defined as small for gestational age (SGA). SGA is defined as a birth weight and/or length that is 2 standard deviations or below the population mean, therefore 2.5% of newborn babies will meet the definition. IUGR may or may not result in a SGA birth weight. Similarly, a baby may be born SGA with IUGR Figure 1.1. SGA may result from varied underlying aetiologies and is not a diagnosis in itself. However, importantly, 90% of children born SGA will show spontaneous catch up growth (19), which differs from SRS.

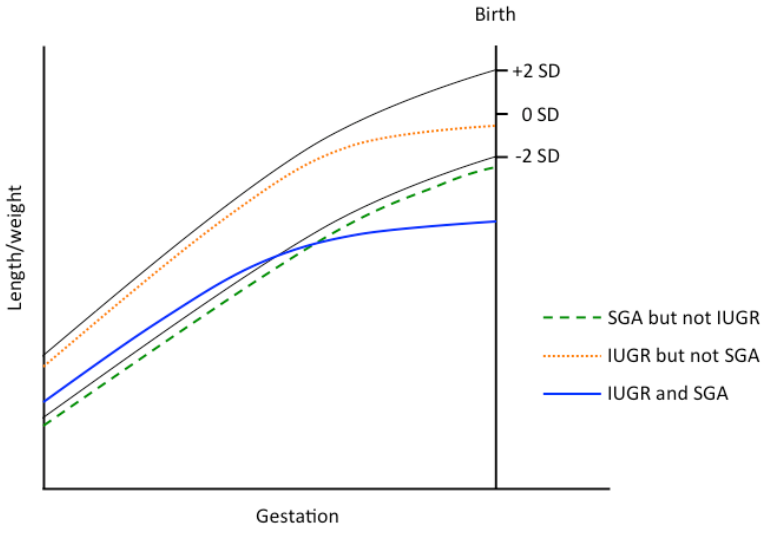


Figure 1.1. Length/weight against gestation prior to birth in IUGR and SGA.

In SRS, IUGR has been proposed because of the absence of familial growth abnormalities, histories of 'threatened abortion' in the first trimester of pregnancy and 'small, infarcted' placentas suggesting a pathological process. This reported IUGR was suggested as early as at three months gestation (2) however, it is not possible to confirm the exact growth pattern as serial measurements were not reported. In a cohort of clinically and molecularly diagnosed patients, there was a positive maternal history of reduced growth in utero in 29/50 cases of clinically diagnosed SRS and in 11 cases this was reported before 26 weeks' gestation (20). In a study of 64 molecularly confirmed cases, reported 'average' gestational age for detection of IUGR was 23 weeks and placental abnormality was observed in 27% of all cases (21). However, the

routine timing of fetal anomaly scans in pregnancy is likely to affect the gestational age at which poor growth is detected and the lack of routine fetal growth monitoring in early pregnancy makes it difficult to definitively establish the growth pattern in the first trimester. The reduced fetal growth seen in SRS at 23 weeks may reflect a constant growth rate from a constitutionally small fetus, resulting in SGA without IUGR. Alternatively, IUGR may occur and precede routine ultrasound scans therefore the precise timing of onset is unknown although SGA is usually observed in SRS. Associated oligohydramnios has also been reported (22).

In many early reports, SRS was grouped within other syndromes causing babies to be born SGA and this makes interpretation of the clinical findings difficult. However, more latterly, studies based on predominantly clinical diagnoses have estimated that the mean birth weight SDS in SRS ranges from -2.94 (20) to -2.65 (23) and does not correlate with maternal height (20). However, in molecularly confirmed cohorts mean birth weight SDS has been reported as -3.4 (SD ± 0.7) (24).

There may be a propensity towards intervention during pregnancy as a result of concern regarding fetal growth. This is supported by the finding that in 8 of 50 cases, an elective decision was made to deliver preterm (20). However, in another report 68% (17/25) of babies were born between 38 and 42 weeks' gestation (23); 20% (5/35) between 36 and 38 weeks; and 12% (3/25) born before 36 weeks. Furthermore, a study reported mean gestational age (calculated in weeks of amenorrhoea) of 37.6±2.6 in 39 molecularly confirmed cases of SRS (24). The method of delivery was not reported and it was not clear whether the observed outcomes resulted from spontaneous onset of labour or medical intervention.

In this study, the history of participants' mothers' pregnancies will be taken retrospectively. Birth weights, which are generally available, will allow assessment for SGA. Estimation of the gestation at which IUGR occurs in SRS might improve identification of affected fetuses and/or suggest causative mechanisms.

1.2.1.2 Short stature

In historical cohorts, which can be useful as they present data on untreated patients, the mean height standard deviation score (SDS) at diagnosis or referral in clinically diagnosed SRS was between -3.58 (14) and -3.45 (23). Mean height SDS of -3.61 and -3.58 in girls and boys respectively were reported in 18 patients approaching final height (25). Seven patients aged above 18 years at assessment in the clinically diagnosed cohort of 50 reported by Price, had a mean height SDS of -3.25 (20). A study of 386 clinically diagnosed cases showed mean adult heights in men and women of 151.2 cm (-3.7 SDS) and 139.7 cm (-4.2 SDS) respectively (26).

More recently, in children with molecularly confirmed SRS weight has been shown to be affected to greater degree than length with a mean weight SDS of -4.3 (27) and mean length SDS between -3.7 (24) and -3.3 (27). Postnatal catch-up growth (without treatment) has been described, although typically short stature is present through childhood, and continues into adulthood (21). These findings consistently meet the definition of short stature (height <-2 SDS) and assist in the evaluation of growth patterns for compatibility with SRS.

Bone age is often delayed early in childhood but this catches up later in childhood (28). Acceleration in bone age has been estimated to occur at age 10 years (26). The underlying pathophysiology has not yet been elucidated. However, early adrenarche and early onset of puberty with a faster tempo and reduced pubertal growth spurt and compromised final height have been proposed (15). Another study demonstrated adrenarche was accelerated by approximately one year in both sexes and that premature adrenarche occurred more frequently than the general population but was contradictory in that no correlation was found between age at adrenarche and adult height (29). Understanding bone maturation in SRS requires further research and might represent a means to maximise height final height.

1.2.1.3 Asymmetry

Asymmetry was frequently described in early cohorts with a prevalence of 33-60% of patients (18, 20, 30). Asymmetry of both upper and lower limbs was reported in 20-60% (20, 31) and leg length discrepancy alone in 14-32% (20, 31) of clinically diagnosed SRS cases. More recently, asymmetry has been reported in 56% of a molecularly confirmed SRS cohort (21). This is a useful distinguishing feature of SRS compared to alternative causes of short stature. Asymmetry is an important diagnostic feature of SRS (15, 24, 32) which may have clinical utility in adults as well as children although it is not specific.

1.2.1.4 Relative macrocephaly

The triangular facial shape in SRS results from a broad forehead due to sparing of brain growth in the skull vault, with the lower part of the face appearing small with a pointed chin as a result of reduced growth. Relatively preserved head growth and the subsequent apparently large head size in comparison to length/height is termed ‘relative macrocephaly’ and has been demonstrated in several studies (21, 24, 33). Normal occipito-frontal circumference (OFC) (mean SDS -1.27) was reported in the clinical cohort of Lai et al however there was a wide range of results (SD 1.73) (23). Price et al reported the mean height, weight and OFC SDS of patients aged above 18 years at assessment: these were -3.25, -2.75 and -2.00 respectively showing a difference in SDS of 1.25

between height and OFC (20). Relative macrocephaly was present in 70% of cases of the Abu-Amero cohort (33). Both Netchine et al (24) and Bartholdi et al (34) defined relative macrocephaly as birth OFC (occipito-frontal diameter in the latter description) SDS ≥1.5 above birth weight and/or length SDS. The former group found this degree of relative macrocephaly in 84.6% of their clinical cases and 96% of molecularly confirmed cases (24). In the Wakeling et al cohort head circumference SDS ≥1 above length SDS, was reported in 76% of molecularly confirmed patients aged 0.8 to 26.8 years (21). Dias et al suggested a definition of OFC SDS >1.5 above height SDS (35). Most recently, the Netchine-Harbison clinical scoring system proposed that relative macrocephaly is present at birth when the OFC SDS is ≥ 1.5 above the birth weight and/or birth length. This was described in 82.1% of patients who met their clinical criteria and 81.8-96.9% of molecularly confirmed SRS cases (32). This definition has been agreed by international consensus (15). It is important to highlight that the absolute OFC may be below the normal range and still show relative macrocephaly in comparison with height.

Relative macrocephaly is an important diagnostic feature of SRS as it can be used to exclude causes of poor in-utero growth which are accompanied by impaired brain growth (e.g. chromosome copy number changes). Evaluation of OFC in adults with SRS may reveal a similar or contrasting pattern to childhood SRS and will be compared in the adult phenotype.

1.2.2 Dysmorphology

Of the clinical cohort described by Lai et al, 92% had the facial features of a small, triangular face, relatively large forehead, a small chin, downturned corners of the mouth, and low-set ears (23). Molecularly confirmed SRS cases have been reported to demonstrate frontal bossing in 60%, micro-/retrognathia in 55%, down-turned corners of the mouth in 27%, and low-set and/or posteriorly rotated ears in 48% (21). Other features of SRS including, blue sclerae and low set ears were described historically (18, 36). With increasing age, the facial dysmorphism is less apparent: females develop a more round face and in males jaw growth accounts for this (20, 21). Abnormal growth of eyebrows, full and prominent eyelashes, epicanthic folds and unilateral ptosis have been reported in clinically diagnosed SRS cases (37). Iris coloboma has been reported in two molecularly confirmed SRS cases (21, 38).

The palate has been reported to be narrow and high-arched (18) and cleft palate has been reported in both clinically diagnosed SRS cohorts (20, 39) and in 2/64 molecularly confirmed cases (21). The latter series included a patient with a bifid uvula and it could be the same patient who was reported in both studies from the UK making it a less certain that this is an associated feature

(20, 21). The prevalence of cafe-au-lait macules was 4-19% of clinical SRS cases (20, 40) and 14% of molecularly confirmed SRS cases (21).

The description of dysmorphology in SRS has arisen from childhood reports and facial features become less evident with increasing age (21). The adult facial features of SRS are not well documented in the literature. In evaluating the adult phenotype of SRS, the studies described in this thesis will include facial features, which might assist in diagnosing adults with SRS.

1.2.3 Genital anomalies

Genital abnormalities were reported frequently in males in historical cohorts of SRS (18, 36) and include cryptorchidism (20) and hypospadias (18, 21, 36). In the mixed clinical cohort studied by Price et al 52% (n=13) of males required genital surgery; one for hypospadias, four for hernia repair, and eight for undescended testes (20). In a molecularly confirmed cohort, the prevalence of bilateral undescended testes and hypospadias in male patients were 11.1% and 3.7% respectively and the overall prevalence of male genital anomalies was 18.5% (21).

One female in a mixed clinical cohort of 50 patients had a bicornuate uterus (20). A patient with molecularly confirmed SRS has been described with ambiguous genitalia, involving cliteromegaly. Subsequent absent breast development was reported in association with hypergonadotrophic hypogonadism and a hypoplastic uterus and absent ovaries were demonstrated on imaging (38). Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome is characterised by congenital hypoplasia or aplasia of the uterus and upper part of the vagina in females with a normal karyotype and otherwise normal secondary sexual development. Two molecularly confirmed cases of SRS have been described with clinical features compatible with MRKH syndrome; a hypoplastic uterus in one and absence of the uterus and upper vagina in the other (41) and the diagnosis has formally been made in two patients who had clinical diagnoses of SRS (42, 43).

Ambiguous genitalia were reported in historical reports of SRS (44, 45) and one patient in a clinical cohort of 50 (20). In molecularly confirmed cases, unilateral and bilateral anorchia and cryptorchidism have been reported (41).

Although the frequency of male genital anomalies has been described in one series (21), the overall prevalence of male and female genital anomalies in SRS is not clear. The frequency of genital anomalies will form part of the description of the adults in this study and fertility in adults of both sexes will be determined where possible.

1.3 Puberty in SRS

Elevated urinary gonadotrophins were reported in very early cases of SRS (1) however this finding has not been reproduced and apart from one case (38) gonadal failure is not a recognised feature of SRS. Precocious puberty has been described in less than 10% of clinical SRS cases (26) but was not reported in a cohort which included a high proportion of molecularly confirmed cases (46). By contrast, early adrenarche has been reported in molecularly confirmed SRS with an estimated bone age advancement of approximately one year (29). In a clinically diagnosed SRS cohort, puberty onset was reported at median ages of 10.3 years (range 6.6-12.1) and 12.1 years (10.6-15.1) in girls and boys respectively (47). In a mixed cohort of clinically diagnosed and molecularly confirmed SRS cases, the median ages at the start of puberty were very similar: 11.8 years (IQR 11.0-12.5) in boys and 10.0 years (9.5-10.5) in girls (29).

In this study, historical puberty onset will be reviewed in a molecularly confirmed cohort with the aim of increasing the understanding of the timing of puberty in SRS, which might indicate potential clinical effectiveness of treatments to delay puberty.

1.4 Feeding and gastrointestinal diagnoses

Poor feeding is frequently described from infancy, with poor breastfeeding and/or suckling. The requirement for nasogastric feeding was described in a historical case of 'severe Silver-Russell syndrome' (36). A study of clinical SRS cases demonstrated that 80% (20/25) experienced 'major feeding problems' which lead to poor weight gain in infancy or childhood (23). 58% (29/50) of a mixed clinical and molecularly diagnosed cohort were reported to require admission to a special care unit with the majority requiring nasogastric tube feeding (20). The same study reported that 56% of parents experienced difficulty feeding their children, which sometimes necessitated hospital admission and that children demonstrated reduced interest in food and required frequent, small feeds (20). A feeding assessment questionnaire has been administered in children aged 2-11 years with clinically-diagnosed SRS. The results showed that children with SRS had increased prevalence of feeding problems, negative mealtimes, food refusal and food fussiness than a control group with no growth problems. However, a three-day food diary showed the children with SRS did not consume significantly fewer calories, carbohydrates, fat or protein than the control group (4781 kcal (SD 1166.7) in the SRS group and 5358 kcal (SD 1066.5) in the control group) (48).

Feeding difficulties have been reported in 67-84% of patients with molecular confirmation of SRS which was thought to result in reduced enteral intake (24, 27). Malnutrition (defined as weight/expected-weight-for-height ratio <80%) has been described in 70% of children with SRS and a body mass index (BMI) SDS of <-2 was reported in 61% of the same cohort (27). The correction of calorific intake to 'adequate' levels has been highlighted as extremely important and authors have acknowledged that supplementary enteral feeding via nasogastric or gastrostomy tube may be required (15). It is possible that low calorific requirement is related to small size, however a reduction in weight SDS compared to height SDS has been reported (27) suggesting a deficit of weight gain. The need for enteral feeding has been described in 30% of molecularly confirmed cases with the majority (72%) of these undergoing gastrostomy insertion. Amelioration of feeding difficulties has been reported to occur after age three years, although this may have been confounded by growth hormone (GH) treatment received (27).

Gastro-intestinal disorders have been reported in SRS and may exacerbate feeding difficulties. These include constipation, gastro-oesophageal reflux disease (34%), reflux oesophagitis (25%) and gut dysmotility with Nissen fundoplication performed in 18% (16). In a molecularly confirmed cohort of 75 children the following findings were reported to exceed healthy-population prevalence: constipation in 20%, infant regurgitation in 50% and persistent vomiting over the age of one year in 29% (27). The potential requirement for gastro-jejunal tube feeding or Nissen fundoplication has been recognised (49). Nissen fundoplication has been reported in 10% of children with molecularly confirmed SRS in France (27).

In summary, feeding difficulties are marked during infancy and childhood and may be accompanied by gastro-intestinal disorders. There is some suggestion of improvement during childhood, though this is variable and there are currently no reports on feeding behaviour in adults. Eating patterns are likely to affect the body composition of adults with SRS.

1.5 Metabolic abnormalities in SRS

1.5.1 Hypoglycaemia

Excessive perspiration in association with both tachypnoea and hypoglycaemia in the neonatal period were described is historical reports (45, 50). Diaphoresis and pale episodes during early life were reported by 52% of parents of children with a clinical diagnosis of SRS (20). These were historical reports and not investigated, therefore blood glucose measurements were not available. In a clinical SRS cohort (n=25), hypoglycaemia following a short period of fasting was

reported in 16% and in another 20% there were episodes of drowsiness, lethargy, excessive sweating or irritability which were terminated with feeding but had not been investigated. These episodes were thought to occur more commonly during early childhood but were reported until the study period when the mean age of study participants was 3.72 years (SD 2.32) (23). In a large cohort of molecularly confirmed cases, episodes of excessive sweating were reported in 67% and documented hypoglycaemia in 84% (21). In another of 75 molecularly confirmed SRS cases, 12% experienced recurrent, symptomatic hypoglycaemia (defined as blood glucose concentration <60 mg/dL which corresponds to 3.3 mmol/L) (27).

1.5.2 Metabolic and cardiovascular risk factors

Diabetes with insulin resistance (i.e. type 2 diabetes mellitus) has been described in a clinically diagnosed girl with SRS aged 15 years, who was reported to have a BMI of 16.88kg/m^2, however BMI SDS was not stated (42). Type 2 diabetes mellitus has been reported in a 27-year-old patient with SRS who had a BMI of 29 kg/m^2 (2.1 SDS) which is just below the limit for definition of obesity (BMI ≥30 kg/m^2). This patient was reported to have gained weight excessively from age 20 years onwards (51). However, it is difficult to determine the risks of diabetes in SRS from such case reports.

In a study of seven molecularly confirmed SRS cases aged 18 to 46 years, fasting glucose levels were normal (4.4 to 5.4 mmol/L). However, glucose intolerance and hyperinsulinemia was demonstrated on oral glucose tolerance test in 28.6% (2/7) at ages 18 and 27 years. No cases of diabetes mellitus or metabolic syndrome were diagnosed. Elevated total cholesterol levels were reported in 28.6% (2/7) at ages 26 and 46 years. Elevated LDL cholesterol (4/7;57.1%) and low HDL cholesterol (4/7;57.1%) were also observed. Normal triglyceride levels were reported in all cases. Of the six individuals with reported blood pressure measurements, there were no cases of hypertension (diagnostic criteria: systolic BP ≥135 mmHg and/or diastolic BP ≥85mmHg) (52).

The true prevalence of diabetes mellitus in older patients with SRS is unknown, however this is important to establish as SGA is known to be a risk factor and early diagnosis and treatment might prevent or reduce complications. Furthermore, appropriate lifestyle advice could be given to adolescents with SRS with the aim of primary prevention.

1.6 Associated clinical features and complications of SRS

1.6.1 Skeletal associations

Clinodactyly was reported in 92% of a clinically diagnosed cohort (23) and is a common feature. The prevalence of clinodactyly in molecularly confirmed SRS cases has been reported as 64-66% (21, 24). Syndactyly of second and third toes is described in 22% of confirmed cases (21). Neither skeletal association is specific to SRS but may be useful features to support the diagnosis.

Incomplete supination of the forearm was noted in one of the first descriptions of SRS (2). Other musculoskeletal problems present in SRS and may require intervention. In particular, limb length discrepancy is proposed to progress with increasing age (and may result from evolving slow growth in affected cells in the body) and lengthening surgery may be required in order to equalise limb lengths. When subjected to surgery, comparable lengthening responses have been observed in patients with SRS (diagnosis not specified) compared to a mixed control group (53).

All adult patients with asymmetry, in the mixed cohort of Price et al, had postural scoliosis and reported recurrent back pain (20). However, another study reported a scoliosis prevalence of 36% (9/25) in clinically diagnosed SRS without any complaints of back pain. Scoliosis was thought to compensate for leg length discrepancy in six of these cases (31). More recently, spinal anomalies in SRS were assessed and found to be present in 22% of cases (35/163); scoliosis alone in 15%, kyphosis alone in 3% and kyphoscoliosis in 4% (54). The direction of scoliosis was not associated with the laterality of shorter limb length. Patient-reported management of scoliosis in those 35 patients included 20% brace wear, 9% previous surgery, and 9% planned surgery (54). Hip dysplasia has been reported in 12% (3/25) of clinically diagnosed SRS with two of the three patients requiring operative intervention (31) and in 3% (2/64) of a larger cohort of molecularly confirmed SRS cases (21).

Patients have been described with a unilateral duplicated thumb (20), and bilateral dislocation of the radial heads with limited elbow extension (20). Camptodactyly of all fingers with arthrogryposis of the distal interphalangeal joints was found in 20% (10/50) of patients with the classical facial appearance (20). These findings have since been replicated (21) however it is difficult to establish whether these are the same individuals as both were from UK cohorts. Other reported abnormalities in individual patients clinically diagnosed with SRS are varied and include: pseudoepiphysis, notched metacarpal bone or phalanx, ivory epiphyses, congenital patellar dyslocation, slipped capital femoral epiphysis, pectus carinatum, increased femoral anteversion, vertical talus, cavovarus of the foot, congenital elevated scapula, congenital radial head

dislocation (31), fixed flexion deformities of the fingers, syndactyly of finger webs, trigger finger, swan neck deformities, and cleft hand (55). Skeletal findings in molecularly confirmed SRS cases include flexion deformity of fingers, camptodactyly, luxation of the hip and knee, ulnar deviation of the hand, bilateral pes equinovarus deformity (38), and radial hypoplasia (21).

The majority of the studies discussed above included children as well as adults therefore it is unclear whether or not musculoskeletal problems manifest or progress in adulthood. Asymmetry is a diagnostic criterion in the Netchine-Harbison clinical scoring system for SRS (32). In contrast to other characteristics of SRS which are less apparent in older patients, asymmetry is likely to persist – unless surgically corrected – and could be useful in describing the adult phenotype of SRS. The progression and effects of asymmetry (e.g. scoliosis) with increasing age might affect health and quality of life.

1.6.2 Head and neck associations

Of a cohort of 50 cases with predominantly clinical SRS diagnoses, 26% were referred to otorhinolaryngologists (ear, nose and throat, ENT, surgeons) with recurrent ear infections and possible hearing impairment (20). Dental overcrowding in children with micrognathia and limited jaw opening have been reported, and surgical interventions described including multiple tooth extractions and surgery to lengthen the lower jaw (20).

Following detailed ophthalmological assessment in 18 children with clinically diagnosed SRS, abnormalities were detected in 17. Refractive errors were demonstrated in 11 patients (61%). Small optic discs and increased tortuosity of retinal vessels were reported however, retinal activity was normal. Although no significant difference in ocular dimensions between right and left eyes was demonstrated, anisometropia was present in three children (27.2%), which indicates an asymmetrical pathology. Astigmatism was detected in three children (37).

1.6.3 Renal associations

In a mixed clinically diagnosed and molecularly confirmed cohort of children and adults with SRS, one out of 50 required a pyeloplasty for an unspecified diagnosis (20). The prevalence of renal anomalies is variable with two studies of molecularly confirmed SRS cases reporting 3% (2/64) (21) and 29% (2/7) (38).

1.6.4 Cardiac associations

Pulmonary valve stenosis, atrial septal defect, pulmonary artery hypoplasia, and mesocardia have been described in case reports of patients with SRS (9, 56-58). In patients with molecularly

confirmed SRS, complex cardiac malformations have been described: total anomalous pulmonary venous return has been reported in two patients, with the second case complicated by an atrioventricular septal defect and interrupted aortic arch. Both patients died within 48 hours of birth (22). Cor triatrium sinistrum, meaning division of the left atrium in two, caused by an abnormal fibromuscular structure, has been described in one patient (22). Further findings in molecularly confirmed SRS include ventriculo-septal defects (38), dilated cardiomyopathy (59), Wolff-Parkinson-White syndrome and pulmonary hypertension (51). However, there are no consistent findings. Congenital cardiac defects were reported in 7.8% (5/64) of a molecularly confirmed SRS cohort (21) compared to birth prevalence of ~1% in live births (60).

1.6.5 Neurological associations

Myoclonus-dystonia, a disorder characterised by myoclonic jerks and/or dystonia with childhood onset, has been reported in SRS cases caused by maternal uniparental disomy for chromosome 7 (see section 1.10.5.1). Dystonia is typically mild and myoclonic jerks particularly affect the axial muscles and the arms (21, 61, 62) (see section 1.10.7). The symptoms often start late in childhood and may progress. Clinical signs will be examined for in adults with matUPD7 in this study.

1.7 Development and cognition in SRS

Delayed motor milestones and poor speech development were described in an early clinical case of SRS (36). It is difficult to confirm whether or not historical cases truly describe SRS, however developmental delay has been reported in 15-34% of molecularly confirmed SRS cases in more recent studies (21, 27).

In a historical cohort, early cognitive assessment of children with clinically diagnosed SRS demonstrated that intelligence quotient (IQ) scores were in the normal range; a mean intelligence quotient (IQ) score of 103.4 with a range from 70-130 (14). In a cohort of 25 clinical cases aged 6 to 12 years, the mean IQ score was 85.9, 32% had IQ scores within the range for intellectual disability (<70), and 20% had scores which were in the borderline range (70-84). A significant positive correlation between IQ score and OFC at the time of review was demonstrated, however this was not true of OFC at birth. 36% had formal statements of special educational needs, 12% received additional support without a formal statement and 48% received speech therapy (23). 20% (10/50) in another clinically diagnosed cohort required speech and language therapy and 38% (14/50) required a statement of educational need with four attending a special school (20).

However, of those aged above 5 years, there was no significant difference in mean OFC between those who did and did not require additional help at school and no correlation was found between additional needs and feeding difficulty or history of hypoglycaemic symptoms. The authors suggested that difficulties in reading and writing might not have been detected at younger ages because of infantilisation of children with SRS (20). Autism has been reported in molecularly confirmed SRS (maternal uniparental disomy for chromosome 7) (63).

The developmental delay and/or cognitive difficulties seen in SRS may differ depending on (epi)genetic subgroup (see genotype phenotype correlations in section 1.10.7). This highlights the importance of SRS genomic stratification. Knowledge of the previously reported associations of SRS will assist in understanding compatible features in this study. In describing the adult phenotype, the natural history of associated conditions may be elucidated.

Table 1.1 and Table 1.2 summarise the clinical features of SRS.

Table 1.1 Additional features of SRS. Table adapted from (15).

Clinical feature	**Frequency % (total number of patients)**
Triangular face	94 (164)
Fifth finger clinodactyly	75 (319)
Shoulder dimples	66 (61)
Micrognathia	62 (115)
Low muscle mass	56 (103)
Excessive sweating	54 (106)
Low-set and/or posteriorly rotated ears	49 (266)
Down-turned mouth	48 (176)
High-pitched or squeaky voice	45 (26)
Prominent heels	44 (61)

Clinical feature	**Frequency % (total number of patients)**
Delayed closure of fontanelle	43 (47)
Male genital anomalies	40 (85)
Speech delay	40 (189)
Irregular or crowded teeth	37 (195)
Motor delay	37 (254)
Syndactyly of toes	30 (264)
Hypoglycaemia	22 (104)
Scoliosis and/or kyphosis	18 (227)

Table 1.2 Summary of clinical characteristics of SRS.

Clinical feature	**Results**
Growth patterns	SGA with BW SDS -2.94 to -2.65
Short stature	Height SDS -3.61 to -3.25
Asymmetry	20-60% prevalence
Relative macrocephaly	70-96% prevalence
Dysmorphology	Triangular facies in 94%, low-set and/or posteriorly rotated ears in 48-49%, retro-/micrognathia in 55-62%, fifth finger clinodactyly in 64-92%, down-turned mouth in 48%

Clinical feature	**Results**
Male genital anomalies	18.5-40% prevalence
Puberty onset	Median age in girls 10.0-10.3 years; median age in boys 11.8-12.1
Feeding difficulties	58-84% prevalence

1.8 Body composition in SGA and SRS

1.8.1 Body composition in children born SGA but not specifically SRS

Reduced muscle mass rather than fat mass is found in children with SGA irrespective of cause (64, 65). Reduced grip strength has also been reported (65), suggesting that reduced muscle mass has an effect on muscle function.

Adults who were born SGA have been demonstrated to have higher limb, trunk and total fat mass than healthy adults who were born at appropriate weights for gestational age (66). Adults born SGA who went on to catch-up in their growth have significantly greater fat mass percentage than controls. Childhood weight gain was strongly associated with waist-to-hip ratio and trunk fat-total fat ratio. Lean body mass was significantly determined by birth weight, age at follow-up, gender and adult weight and height (67).

Negative effects on bone mineral content and bone density have been reported in SGA (64, 68) although postnatal growth has been demonstrated to modify this (69, 70).

1.8.2 Body composition in SRS

Relatively few studies have been done on SRS cases distinct from SGA. In historical SRS studies, marked leanness has been observed in infants and young children (2), and skinfold thickness measurements were markedly below normal (14) but became closer to the population mean during adolescence (25).

In a small study of seven molecularly confirmed SRS cases aged 18 to 46 years, all waist circumferences were within normal ranges (≤88 cm in women and ≤102 in men) and waist-to-hip ratios were at the upper limit of normal in males (0.87-0.90; abdominal obesity defined as >0.90 in males) and in the upper range of normal in females (0.66-0.79; abdominal obesity defined

as >0.85 in females). One patient had high waist to hip ratios but was obese (BMI SDS 2.68). Compared to normative data, the cases displayed high fat mass percentage (38.2%, SD 10.2, range 26-55.7), high fat mass index (mean 8.37 kg/m^2, SD 4.47 range 4-17.4), high trunk/limb fat ratio (mean 0.93, SD 0.45; range 0.24-1.73) and low lean body mass (mean 25.84kg, SD 2.16, range 21.7-28.5). Bone mineral density was in the normal range with a mean spine Z-score of 0.1 (SD 1.2) and a mean total body Z-score of 0.44 (SD 0.9) (52).

1.9 Long-term effects of SRS

1.9.1 The life course of SGA

There is a body of evidence available on the effects of birth weight and predisposition to disease in later life. An inverse correlation has been described between birth weight and non-communicable diseases like ischaemic heart disease, and increased early mortality. The same relationship has been demonstrated with weight at one year. Hypertension was linked to low birth weight but not weight at one year (71, 72). These results suggested that both prenatal and postnatal growth are important factors in adult health. It is important to note that the first epidemiological study included males only and 92.4% were breastfed. Therefore, the wider applicability of these findings to a mixed population and to formula-fed infants and children may not be appropriate. However, fetal and infant growth in women was evaluated subsequently, and a decreased risk of death from cardiovascular disease was associated with increasing birth weight. The previous correlation with weight at one year and cardiovascular disease was not demonstrated (73).

Another epidemiological study evaluated the effect of infant feeding on serum cholesterol concentration and death from ischaemic heart disease. There was a negative correlation between birth weight in all feeding groups and risk of death from ischaemic heart disease. The effect was greater when weight at one year was assessed. The lowest risk of ischaemic heart disease was found in mixed fed infants, followed by those who were exclusively breast fed (lower risk if weaned at one year) and the highest risk was found in exclusively bottle-fed infants. Those who were mixed or breastfed and weaned at one year showed no correlations between cholesterol level and birth weight or weight at one year. However, breast fed infants who were not weaned at one year, showed a positive correlation between birth weight and both increased risk of ischaemic heart disease and serum concentrations of total cholesterol and LDL cholesterol but no difference in HDL cholesterol or triglycerides. This group also demonstrated a positive correlation between weight at one year and both cholesterol level and birth weight. A

comparable social class at follow-up was reported, although there was over-representation of lower social class in the prolonged breastfeeding group. This might be expected for financial reasons and the authors suggested as a means of contraception. Lifestyle and cardiovascular risk factors at the time of follow-up would be important. As expected, there was a positive correlation was between cholesterol level and both BMI and waist-to-hip ratio. However, adjustment for these did not remove the trends described above (74).

Impaired glucose tolerance or diabetes has also been associated with men and women who had lower birth weight and weight at one year. In both genders, this finding was independent of body mass at follow-up, although increased weight and BMI at follow-up were reported in the cases with abnormal glucose control. In both men and women systolic blood pressure was noted to reduce with increasing birth weight and in men this also correlated with weight at one year (75, 76).

The theory of 'programming' refers to the idea that changes in the environment during critical points in the early development of an organism cause long-term or permanent physiological or structural changes to tissues or organs. The 'Barker Hypothesis' is now a widely-accepted theory that an environment of under-nutrition and poor growth during fetal, and postnatal life causes not only reduced body size, but changes in body composition and altered hormonal axes, which persist into adult life and manifest as risk factors for cardiovascular disease (73). The described associations are independent of alcohol, smoking and obesity but may be exacerbated by life style factors (77); the developmental origins of adult health and disease hypothesis (the 'DoHAD' hypothesis) (78). The developmental origins of insulin resistance have been described in adults (79).

1.9.2 The life course of SRS

There is extremely limited published evidence on long-term effects, adult outcomes and life expectancy in SRS. In the absence of life threatening congenital malformations, life expectancy is not believed to be reduced. Owing to low birth weight, adults with SRS may be at increased risk of cardiovascular disease for the same reasons as any adult born SGA. The impact of the DoHAD hypothesis (see section 1.9.1) is likely to be of significance to people with SRS given that they are also born SGA. However, the differing patterns of growth in SRS may result in specific adult outcomes.

Few case reports of adults with SRS have been published. A 32-year-old woman who presented with a clinical diagnosis of SRS and dilated cardiomyopathy was reported to have a history of

systemic hypertension (59). Three case reports of patients aged between 18 and 27 years with genetic confirmation of SRS have further raised concerns about the adult health of individuals with SRS (51). One patient was reported to have gained excessive weight at approximately age 20 years and at the time of the report had a BMI of 29 kg/m^2 (SDS 2.1), as well as type 2 diabetes mellitus and microalbuminuria. The second patient was found to have raised low-density lipoprotein (LDL) cholesterol and total cholesterol levels, elevated alanine aminotransferase levels with fatty liver infiltration, microalbuminuria, and high glucose and HbA1c levels. The third patient had previously been treated for glomerulonephritis and later developed hypertension, with microalbuminuria and raised uric acid.

As previously discussed, in two cohorts, including molecularly confirmed and clinical diagnosed cases, adult height was significantly reduced (SDS ranged from -4.3 to -1.4) with head circumference affected to a lesser degree (SDS of -1.9 to 2.3) (52) but there is limited information on medical issues.

A larger study compared 29 individuals with SRS (including 20 molecularly confirmed cases) to non-SRS SGA cases. The mean age of individuals with SRS was 18.3 years (SD 1.6). Mean height SDS at GH start was -3.6 (SD 0.8) and head circumference SDS was -1.73 (SD 1.5). The mean height SDS at adult height was 1.63 (SD 0.8) and mean head circumference SDS -0.64 (1.1) (80). This study also examined metabolic health (see section 1.13.3.2) and body composition (see section 1.13.3.1) during and for two years following GH treatment.

There are no publications on pregnancy or educational attainment in a large cohort and none that record the lived experience of patients. Therefore, medical practitioners are currently unable to provide clear information on prognosis for children newly diagnosed with SRS. This study aims to gather useful evidence.

SRS is primarily a clinical diagnosis and this chapter has highlighted the major clinical features of SRS, describing the growth pattern, dysmorphic features, associated conditions, and marked feeding difficulties. However, the SRS phenotype described so far relates predominantly to children.

1.10 Genetic mechanisms causing SRS

1.10.1 Overview of DNA and chromosomes

Before discussing the molecular genetic mechanisms in SRS, an overview of deoxyribonucleic acid

(DNA) will be discussed. DNA is comprised of bases, sugars and phosphate molecules. Deoxyribose and phosphate molecules are sequentially connected via phosphodiester bonds and make up the backbone. The bases point inwards and their linear sequence constitutes the genetic code. The nucleic bases in DNA are adenine (A), guanine (G), cytosine (C) and thymine (T). Bases are made from rings of nitrogen and carbon atoms and are classified into purines (A & G), which comprise two interlocked rings and pyrimidines (T & C) which have only one ring. The basic subunits of DNA are nucleotides which include one deoxyribose, one phosphate and one base. DNA is generally found in a double-stranded state with the bases pairing together in a specific manner; A with T and C with G. This produces the anti-parallel, double-helical structure of DNA, which is stabilized by hydrogen bonds between the paired bases.

DNA is organised into chromosomes, which constitute discrete units of DNA sequence to enable DNA in the nucleus to be organised effectively, particularly during cell division. It was established, in the 1950s, that humans have 46 chromosomes. These include 22 autosomal chromosomes that are inherited in duplicate; one from each parent. The sex chromosomes are inherited asymmetrically since the mother always passes on an X chromosome to her offspring while the father can pass on either an X or a Y chromosome.

The double helix of DNA within each chromosome is packaged along with basic proteins (called histones) into units called nucleosomes. Each nucleosome comprises 146 base pairs (bp) of DNA wrapped just under two turns around a core of eight histones. Nucleosomes are organized into a 30 nm fibre through the addition of more histones. Chromatin describes the three-dimensional packing of DNA and protein, which influences its accessibility and function. Euchromatin is formed with nucleosomes packaged loosely and therefore accessible to other proteins and can be actively transcribed. Heterochromatin is the densely packed inactive form. Recognising the importance of molecules that associate with DNA is very important to the pathogenesis of SRS.

In each cell division, the double-helix is unwound and DNA is copied by the DNA polymerase machinery so that all genetic material is doubled and subsequently divided between the two new daughter cells. In addition to the storage and passage of genetic information from one cell to the daughter cell, DNA is vital for cell function. Portions of DNA serve as templates for the creation of ribonucleic acid (RNA) molecules by RNA polymerase in a process called ‘transcription’. A section of DNA sequence, with a specific location on a chromosome, which acts as a template for the production of a RNA sequence is called ‘a gene’. RNA is similar to DNA in having a sugar and phosphate backbone, however it differs in several ways: a) the sugar in RNA is ribose rather than deoxyribose; b) the base uracil (U) is present in place of thymine; c) RNA is usually present as a

single-stranded structure. There are different types of RNA including non-coding RNA molecules. Messenger RNA functions as a template for protein production in a process termed 'translation'.

The expression of genes can be modified by multiple genetic mechanisms, many of which affect DNA sequence such as deletion, duplication or rearrangement of the DNA bases. However, epigenetic processes also affect gene expression but without altering the DNA – genetic – sequence. The term 'epigenetic' originates from the Greek prefix 'epi-' meaning 'on', 'upon', 'above', 'over' or 'in addition to'. Epigenetic processes, such as methylation of DNA and acetylation, methylation and phosphorylation of histones, modify DNA such that transcription is affected. Epigenetic modifications affect DNA configuration – i.e. heterochromatic or euchromatic states thus altering gene expression and can be passed on during mitosis. Altered epigenetic states can cause disease through altered gene expression as is the case in SRS (see section 1.10.5).

1.10.2 DNA methylation mechanisms

DNA methylation is an important epigenetic mechanism. The chemical modification of DNA involves the covalent addition of a methyl group to cytosine (Figure 1.2). Methylation predominantly occurs at the CpG dinucleotide (cytosine connected to guanine by a phosphodiester bond).

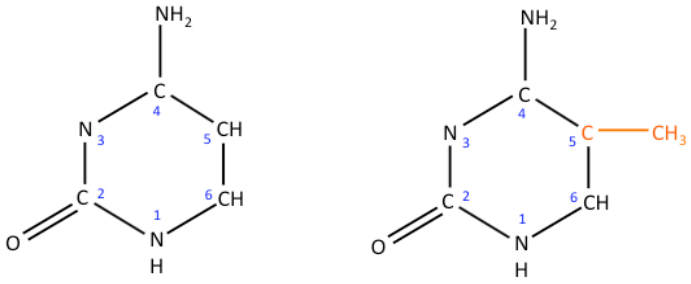


Figure 1.2 DNA methylation. On the left, cytosine is depicted and on the right, the carbon atom at position 5 has been methylated to form 5-methylcytosine (5MeC).

The DNA methyltransferases (DNMTs) are a group of enzymes responsible for DNA methylation. Among this family, DNMT1 maintains methylation during cell division; DNMT3A and DNMT3B

initiate new methylation marks. The process to determine methylation status at any one locus is poorly understood but its presence alters the secondary structure of DNA and can affect gene expression. DNA methylation affects histone modifications and chromatin structure and is a useful marker of the epigenetics state of a locus.

DNA methylation patterns are markedly altered in the developing germ cells and subsequently after an oocyte is fertilised by a sperm cell during zygotic development to a fetus. Methylation is shown in this diagram (Figure 1.3) for the mouse but is thought to be similar for the human. There is loss of methylation in the early germ cell in both males and females as the cells begin afresh. Methylation increases as the egg and sperm mature such that genes required for function are expressed whilst others are switched off. A different subset of genes is expressed following fertilisation as the embryo differentiates into its tissues so there is loss of germ cell methylation followed by gain of DNA methylation in the embryo and fetus. A subset of genes retain their germ-cell epigenetic profile in the embryo and subsequently; these are called imprinted genes. The DNA methylation pattern at imprinted loci observed even in an adult represent the pattern laid down in the egg and sperm that went on to form the individual.

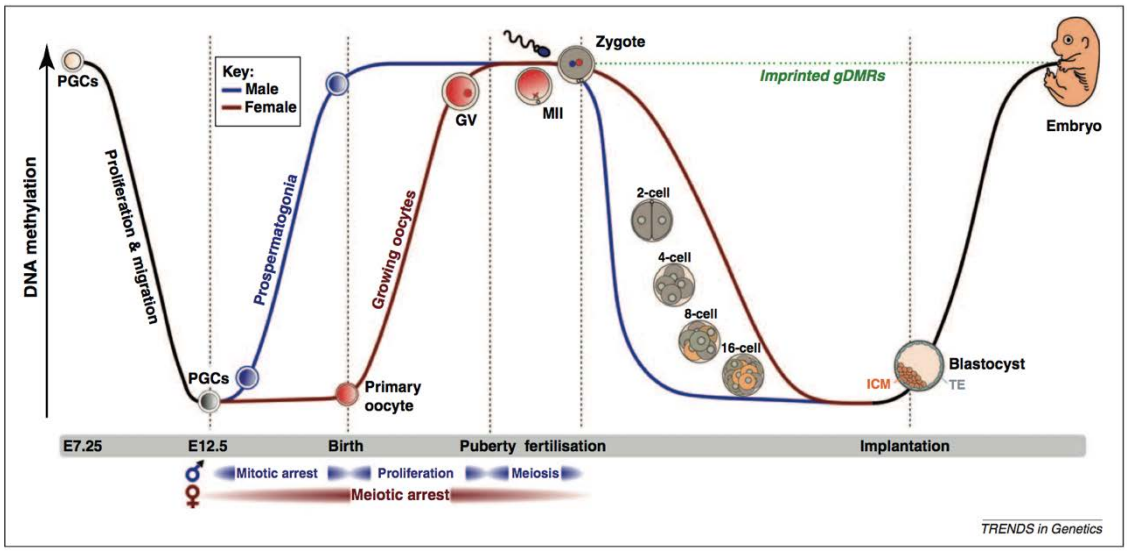


Figure 1.3 DNA methylation changes during early development; from the primordial germ cell to the embryo. Reprinted from Trends in Genetics, Volume 28, Number 1, Smallwood and Kelsey, De novo DNA methylation: a germ cell perspective, pages 33-42, Copyright (2012), with permission from Elsevier. This diagram depicts embryo development in the mouse. A similar process is believed to occur in humans. DNA = deoxyribonucleic acid, PGCs = primordial germ cells, GV = germinal vesicle, MII = second meiotic division, gDMRs = germline differentially methylated regions, ICM =

inner cell mass, TE = trophectoderm, E7.25 = embryonic day 7.25, E12.5 = embryonic day 12.5.

1.10.3 Mechanisms of imprinting

Imprinting refers to the epigenetic processes by which gene expression is dependent on the parent of origin. Differential contributions of the maternal and paternal genomes was demonstrated by the work of Barton et al (81) and McGrath and Solter (82). Their experiments produced gynogenetic (completely maternal genome) and androgenetic (completely paternal genome) mouse embryos and proved that maternal and paternal genomes contributed differently to fetal growth and development and a contribution from both are required for normal development in the mouse. Analogous human examples exist showing these processes: a complete hydatidiform mole contains only paternal DNA; ovarian teratomas only maternal.

In contrast to other genes that are active from both maternal and paternal chromosomes, the expression of imprinted genes may be regulated at specific developmental periods and locations by the parental origin of an allele. A very small proportion of genes are imprinted – approximately 100 are currently recognised (83). Imprinted genes are functionally haploid – meaning one copy is silenced and only one copy is required for normal function, which is a normal process in mammals.

A key type of primary epigenetic mark responsible for the control of allelic expression is DNA methylation. In differentially methylated regions (DMRs) a difference in methylation is observed between the maternally and paternally inherited chromosomes such that one parental copy is methylated and the other not. Primary DMRs have been identified in the majority of imprinted regions of the genome and are associated with various molecular mechanisms that result in imprinted genes within their influence being silenced on one allele and active on the other. As illustrated in Figure 1.3, primary germline imprinted DMRs are reset and established during the production of gametes (84) but maintained after zygote formation. These regions are spared from reprogramming in the wave of demethylation in the early embryo.

1.10.4 Imprinting disorders

Imprinting disorders (IDs) in humans are caused by aberrant imprinted gene expression. Silver-Russell syndrome is one such ID and seven other humans IDs are well-described. These are: Beckwith-Wiedemann syndrome, Prader-Willi syndrome, Angelman syndrome, Temple syndrome,

Kagami-Ogata syndrome, Transient neonatal diabetes type 1 and Pseudohypoparathyroidism type 1b. A novel human imprinting disorder has recently been described: maternal uniparental disomy for chromosome 20 which is characterised by IUGR and faltering growth with marked feeding difficulties (85).

As discussed above, normal imprinted genes are functionally haploid therefore disorders can arise through any mechanism that alters expression of the genes. There are four main molecular mechanisms (Figure 1.4) that alter imprinted gene dosage: 1) chromosomal error such as uniparental disomy (the inheritance of both chromosomes from one parent with no contribution from the other); 2) copy number change such as gene duplication or deletion within an imprinted region with the effect depending on whether it occurs on the maternal or paternal allele; 3) epigenetic change – loss or gain of DNA methylation and no underlying DNA sequence change; and 4) mutation of expressed imprinted genes (also with a parent of origin effect) i.e. mutations only cause disease if the gene is actually expressed and so the clinical impact depends on which parent passes on the mutation). These changes either cause the loss of expression or overexpression of a normally expressed imprinted gene.

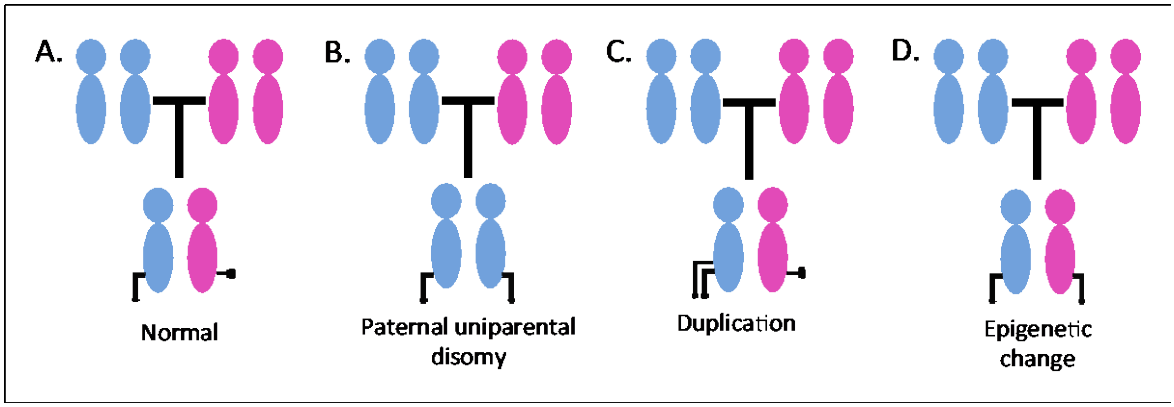


Figure 1.4 Molecular mechanisms affecting imprinted gene dosage. Paternal alleles demonstrated in blue and maternal in pink. A. depicts normal expression from the paternal allele of a given imprinted locus and maternal silencing with a methyl group represented (as a black lollipop). B. shows paternal uniparental disomy and the resultant bi-allelic expression of the imprinted locus. C. demonstrates a copy number change with duplication of the imprinted locus and overexpression. D. illustrates loss of methylation of the maternal locus and aberrant expression in addition to appropriate paternal expression.

1.10.5 Abnormalities of imprinting in SRS

1.10.5.1 Maternal uniparental disomy for chromosome 7

Uniparental disomy (UPD) occurs when both copies of a specific chromosome are inherited from one parent rather than one from each parent (86). After it was noted that UPD of certain particular chromosomes caused intra-uterine growth retardation in mice, this mechanism was suggested as a cause for SRS (87). Because imprinted genes are expressed depending on the parental origin, UPD may cause the absence of an active copy on the parental homologue that is not present. If the active parental homologue is duplicated then there is overexpression of the imprinted gene(s).

The observation of unexpected short stature in human diseases associated with chromosome 7 led to evidence supporting UPD of chromosome 7 as a cause of growth disturbance: two cases with marked short stature also had cystic fibrosis caused by the inheritance of homozygous maternal *CFTR* mutations only present in the mother of the child (88, 89): a third case with a collagen disorder showed homozygosity for a maternal *COL1A2* mutation (90). These are autosomal recessively inherited conditions therefore both the mother and the father would be expected to be carriers of the relevant mutation. UPD was identified because of the genetic investigations for the specific recessive disorders.

Kotzot et al (1995) demonstrated uniparental disomy of chromosome 7 in three of 25 patients with SRS and one case of 'primordial growth retardation' (91). Preece et al (1999) investigated five patients with matUPD7 by examining the complete length of both copies of chromosome 7 (92). Both isodisomy and heterodisomy were found but there was no common region of homozygosity, which made recessive mutations as the cause of SRS unlikely (coupled to the fact that there are very few siblings reported with this condition). However, there are three imprinted loci on chromosome 7 and it is still not clear whether or not SRS is due to a combination of altered expression of genes at all loci or one of them as no mutations or epigenetic errors at any locus has consistently been reported in SRS cases. *PEG1/MEST* (paternally expressed 1; mesoderm expressed transcript) is a paternally expressed gene located at 7q31-34 (93). *GRB10* (growth factor receptor bound protein 10) is a good candidate at 7p11.2 as it binds to the insulin and growth factor receptor, IGF1R (94). It is maternally expressed in some tissues and paternally expressed in others and functions to diminish tyrosine kinase activity which is vital for the growth-promoting activities of insulin (95). *SGCE* (ε-sarcoglycan) gene is expressed on the paternal allele and located at 7q21.3. Loss of expression is associated with myoclonus-dystonia (96, 97) and risk of myoclonus-dystonia specifically in SRS (61, 62).

Maternal uniparental disomy for chromosome 7 has subsequently been identified by many authors and is now estimated to account for 5-10% of SRS cases (20, 34, 98, 99) and is routinely tested for in normal clinical practice when SRS is suspected (15).

1.10.5.2 Abnormalities of chromosome 11

The association between SRS and chromosome 11 became evident by identifying SRS patients who had chromosome anomalies affecting the 11p15 region. Kosoki et al (2000) first described a patient with features of SRS who was found to have a duplication of 11p15 of maternal origin (100). Fisher et al (2002) subsequently identified three patients with growth retardation who had maternally derived 11p duplications (101). One of these patients had a suggested but not definitive diagnosis of SRS. However, it was the discovery of an epigenetic error on chromosome 11p 15 that finally crystallised the association.

1.10.5.3 Imprinted genes at chromosome 11p15.5

Chromosome 11p15.5 region (Figure 1.5) spans approximately 1 Mb and contains two domains of imprinted genes that are each regulated by an imprinting control region (ICR). The domains harbour paternally expressed genes (*IGF2* and *KCNQ1OT1*) and maternally expressed genes (*H19* and *CDKN1C*) which affect placental and fetal growth.

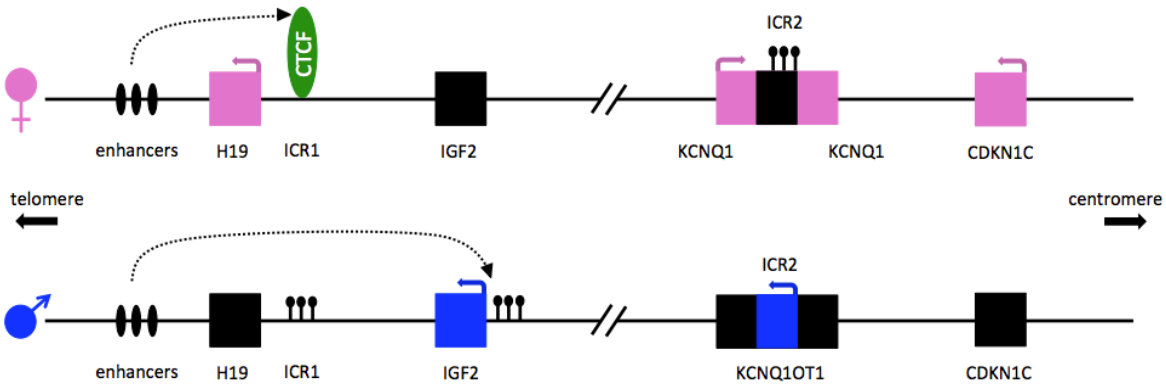


Figure 1.5 The normal 11p15 region depicting the genes *H19*, *IGF2*, *KCNQ1*, *KCNQ1OT1*, and *CDKN1C*, as well as the imprinting control regions (ICRs) 1 and 2. The CCCTF binding factor (CTCF) is also shown. Maternally expressed genes are shown in pink; paternally expressed in blue. Arrows on genes illustrate expression. Genes that are not expressed are black. represents methylation.

ICR1 (also referred to as *H19/IGF2* intergenic differentially methylated region, IG-DMR or *H19* DMR) is telomeric and regulates expression of *IGF2* and *H19*. ICR1 is a paternally methylated germline DMR which is located 2-4 kb upstream of *H19*. It is normally unmethylated on the maternal allele. This was first demonstrated in the mouse *H19* DMR on the syntenic chromosome 7 (102). The paternal ICR1 has been demonstrated to be unmethylated in human fetal spermatogonia but methylated in mature spermatozoa (103). The unmethylated maternal ICR1 is able to bind CCCTF-binding factor (CTCF) for which it has seven binding sites. CTCF is a zinc finger protein that facilitates the conformation of chromatin such that when it binds, the chromatin loop structure is altered to form a boundary which prevents the enhancers downstream of *H19* from interacting with the *Igf2* gene promoter (104). Instead *H19* promoters are activated. CTCF is necessary for prevention of *de novo* methylation at ICR1 on the maternal allele (105). By comparison, on the methylated paternal allele, CTCF is unable to bind therefore enhancers can freely interact with the *IGF2* promoters to cause expression. There are additional secondary DMRs located within this region; a DMR at the H19 promoter and two DMRs at *IGF2*; *IGF2* DMR0 (*IGF2P0*) and *IGF2* DMR2. These are methylated post-zygotically.

Any gain or loss of methylation at ICR1 affects the reciprocal imprinting of *H19* and *IGF2* and results in aberrant expression at the *H19/IGF2* locus. Both genes are expressed in endoderm- and mesoderm-derived tissues during embryonic development and their over- and under-expression are associated with different phenotypes. *IGF2* encodes insulin-like growth factor 2, which is a 7.5 kDa protein hormone. It has a key role in the regulation of fetal growth. *H19* encodes a large non-translated RNA which is expressed during embryogenesis; however, its function is not understood. It has been suggested to limit placental growth (106), implicated as an oncogene (107, 108), and decreased expression has been associated with endometriosis (109). These represent changes in its somatic expression.

The centromeric ICR2 (also known as KvDMR1) is a maternally methylated germline DMR and controls the expression of *CDKN1C* (cyclin-dependent kinase inhibitor 1C), *KCNQ1* (potassium voltage-gated channel KQT-family member 1) and *KCNQ1OT1*. *KCNQ1OT1* is expressed from the paternal allele and is thought to repress both *CDKN1C* and *KCNQ1* expression although some low-level expression from the paternal allele has been described. Methylation of the maternal allele represses expression of *KCNQIOT1* but permits expression of *CDKN1C* and *KCNQ1*. *KCNQ1OT1* overlaps with introns 9 and 10 and exon 10 of the *KCNQ1* gene and encodes another long non-coding RNA. *CDKN1C* gene contains four exons, of which two encode proteins. In

addition to regulation by *KCNQ1OT1*, its expression is also controlled by promoter and enhancer sequences. There is wide expression – in skeletal muscle, heart, lung, kidney, brain, pancreas and testis – and *CDKN1C* negatively regulates cellular proliferation. Gain-of-function mutations in *CDKN1C* have been implicated in IMAGe (intrauterine growth restriction, metaphyseal dysplasia, adrenal hypoplasia congenita and genital anomalies) syndrome (110) which has many clinical features in common with SRS and recently familial SRS was reported where a *CDKN1C* mutation segregated with the phenotype (111) see section 1.10.7.

1.10.5.4 Duplications of chromosome 11p and growth restriction

Large maternal duplications including both ICR1 and ICR2 are generally associated with SRS. The effects of smaller duplications or deletions depends more on the exact content and the parent of origin.

Three cases of growth restriction with maternal 11p15 duplications have been reported (101). The cases all showed prenatal growth retardation and the two surviving cases showed postnatal growth retardation. One was considered to potentially have SRS but did not meet the criteria.

A study of 46 patients with SRS features screened for maternal duplications identified two patients with maternal duplications of 11p15 (at least 5 and 9 Mb) (112). It was assumed that overexpression of the growth restriction gene *CDKN1C* was a key factor in the phenotype. Indeed, a maternal duplication of 11p15 has been reported in another patient with SRS (113) and in this case the duplication of 0.76-1.0 Mb involved ICR2 only and interestingly the patient displayed a severe growth failure phenotype as well as severe developmental delay in addition to the expected features of SRS. It is possible that there were other causes for the severe developmental problems. A maternally inherited duplication of 562-575 kb was found to involve *H19* and ICR1 but not *IGF2* (114) which implies that overexpression of H19 is a factor in the growth phenotype of SRS and is not all related to *CDKN1C* in these duplication cases. The reported clinical features of this patient including growth parameters, frontal bossing, clinodactyly, body asymmetry and feeding difficulties, are consistent with SRS.

1.10.5.5 Maternal uniparental disomy for chromosome 11

As previously discussed, maternal duplication of 11p15 was described in 2000. Prior to that, matUPD7 had been established as a cause of SRS. With knowledge of ICR1 and known mechanisms of imprinting errors, it was postulated that maternal uniparental disomy for chromosome 11 (matUPD11) could be another cause of SRS. This would cause double the normal expression of *H19* and absence of *IGF2* expression.

MatUPD11 was not found in a study of patients who demonstrated either growth retardation or SRS (38). However, mosaic matUPD11 has subsequently been described in a patient with SRS (115). This patient presented with intra-uterine and postnatal growth retardation (birth weight on 0.4th centile; height at age 2 years 6 months -4.5 SDS), mild delay in gross motor development, excessive sweating in infancy and severe feeding difficulties – although the latter may have been compounded by her cleft palate. Additional features included fifth finger clinodactyly, a triangular facial appearance with frontal bossing and slight facial asymmetry, however, there was no limb asymmetry. DNA was extracted from peripheral blood leucocytes only: mosaicism was not demonstrated by physically sampling different tissues but rather inferred from other analyses; methylation-specific polymerase chain reaction (MS-PCR) demonstrated significantly raised methylation at ICR2. Microsatellite analysis for markers from chromosome 11p15.4 to 11p15.5 and 11q13.1 to 11q24.1 were informative and demonstrated increased dosage of the maternal allele. Methylation-specific multiplex ligation dependent probe analysis (MS-MLPA) was also performed and excluded duplications and deletions.

1.10.5.6 Loss of methylation at imprinting control region 1 in SRS

Gicquel et al demonstrated partial loss of methylation (LOM) of the *H19* promoter and ICR1 but no change in methylation at ICR2 in five out of nine patients with SRS (116). Hypomethylation of *H19* has also been described in patients with asymmetry who each had IUGR or postnatal growth retardation. Two of these patients who met diagnostic criteria for SRS as proposed by Price et al, were found to have complete hypomethylation of *H19* (38). H19/IGF2 LOM has subsequently been described by many groups and considered to account for 40-64% of SRS patients (24, 34). Bartholdi et al (34) diagnosed 106 patients with SRS using the authors' criteria and found 27% (n=29) had methylation defects at *H19* and *IGF2*, 9% (n=10) had methylation defects at *H19* and 2% (n=2) had methylation defects at *IGF2* only.

The underlying aetiology of the loss of methylation is unknown in most cases. There have been reports of loss of methylation secondary to genomic deletion; single cases of *de novo* mosaic deletions of *H19* and part of ICR1 (117) and *de novo* mutation in ICR1 causing loss of methylation at CTCF binding sites on the paternal chromosome ((118)) but in most cases the epigenetic defect is therefore believed to be primary i.e. no underlying genetic cause has been identified.

1.10.5.7 Imprinting control region 2 in SRS

As discussed in section 1.10.5.4, a maternally inherited duplication of 11p15 restricted to ICR2 has been described in a case of SRS where there was also marked developmental delay (113). This

duplication would be expected to result in over expression of *CDKN1C* but normal expression of *IGF2* and may explain variation in the phenotype.

Polymorphisms in *CDKN1C* have been described in SRS (119) and more recently a novel mutation of *CDKN1C* (c.836G>T, p.Arg279Leu) has been identified in a family where the proband had SRS with the following clinical features: SGA, poor postnatal growth, relative macrocephaly, low body mass index (BMI), and a prominent forehead during childhood. Her mother and maternal aunt were also born SGA and had short stature with the latter having received GH treatment and reaching a final height SDS of -1.1. Two other female family members presented with severe SGA and poor postnatal growth. All the aforementioned were diagnosed with SRS and carried the same mutation. Although the main features of SRS were present, only one had feeding difficulties and for one patient the only clinical data available was the final height SDS of -3.3. One family member who was born SGA and had short stature but no other features of SRS, was not found to have the mutation. Where SRS was inherited, this was exclusively via the maternal line (111).

ICR2 loss of methylation has been described in association with ICR1 loss of methylation in 4% (3 of 77) (120) and in 4% (3 of 74) cases (121). However, this is neither inevitable nor usually the case as in one further study ICR2 methylation was to be unaffected in 22 patients with loss of methylation at ICR1 (122).

1.10.5.8 Multi-locus imprinting disturbance

In disorders of imprinting, loss of methylation has been described not only at ICR1 but also at multiple other imprinted loci across the genome, a phenomenon termed multi-locus methylation disturbance or multi-locus imprinting disturbance (MLID). MLID has been shown in 9.5% (121) to 11.4% (123) of H19/IGF2 LOM SRS cases and was demonstrated at the other paternally methylated locus *DLK1* at 14q32 as well as *ZAC1* DMR, *PEG/MEST*, *SNRPN*, and *IGF2* DMR20 (121, 123). Turner et al showed that 2/23 SRS cases with H19/IGF2 LOM had methylation loss at other loci across the genome including NESPAS/GNAS and PEG3 DMRs in one case and IGF2R, KCNQ1OT1, PEG10, GRB10 and NESPAS DMRs in the other (124). The causes of these generalised epigenetic errors are not as yet fully understood but it is less likely to be owing to a chromosome 11p 'in-cis' control region rearrangement when multiple loci are affected. Trans-acting variants or environmental/stochastic insults with a global epigenomic effect are more probable causes. ZFP57 mutations have been associated with MLID in transient neonatal diabetes (125). More recently, maternal variants in NLR family, pyrin domain-containing 2 and 7 genes (NLRP2 and NLRP7) and peptidyl arginine deiminase 6 (PADI6) have been identified in MILD associated with SRS (126). These genes encode proteins which are abundantly expressed in oocytes and zygotes.

The variations are suggested to affect the epigenetic features of the offspring and also cause pregnancy loss (126).

1.10.6 Familial Silver-Russell syndrome

Although most cases of SRS are sporadic, families have been described where multiple family members are affected. SRS has been reported in discordant (127) and concordant (128) monozygotic twins. Autosomal recessive, autosomal dominant and X-linked inheritance have been proposed however where these early cases were based on clinical diagnosis alone, confirmation of SRS is difficult.

Two sisters with SRS were described initially by Ounap et al (129) and H19/IGF2 LOM was later confirmed (34). The latter authors also described a brother and sister with classical SRS (34). None of the parents displayed features of SRS. A third family demonstrated dominant inheritance with a father and daughter both clinically affected and confirmed to have epimutations at 11p15 (34). The cause of this transmission in these families is not known but could be due to an imprinting centre mutation or genome rearrangment that has not as yet been demonstrated. More recently, maternal transmission of ICR2 microduplication (130), a *CDKN1C* mutation (111) and IGF2 mutations (131) have been described in familial SRS.

Overall, genetic confirmation of SRS is reported in up to 70% clinical cases. Molecular diagnosis may permit disease stratification, however negative test results cannot exclude the diagnosis of SRS. Research into potential explanations for the remaining 30% of clinical cases is ongoing. Furthermore, the exact imprinted region responsible for matUPD7 SRS has not yet been determined.

1.10.7 Genotype-phenotype correlations in SRS

Although both matUPD7 and H19/IGF2 LOM are important causes of the clinical phenotype of SRS, there is a broad range of clinical features and there is a (epi)genotype-phenotype relationship which is clearer as further cases are studied. Price et al (20) suggested that matUPD7 might be more frequently found in cases not meeting strict diagnostic criteria and the theory of a distinct phenotype in matUPD7 (98, 132) and a more severe phenotype in 11p15 SRS cases has been supported by several authors (21, 34).

Patients with H19/IGF2 LOM have been shown to have significantly lower birth weights than those with SRS and no known (epi)genetic cause (median birth weight SDS -2.85 vs. -1.77, $p<0.05$)(35) as well as when compared to those with matUPD7 (-3.4 vs. -2.6, $p<0.05$)(24). Birth

length has been demonstrated to be lower in 11p15 SRS compared to UPD7 (-3.68 vs. -2.43, p=0.003). A particularly severe subsequent growth failure phenotype has been reported in H19/IGF2 LOM cases (21, 35, 133) in that the degree of hypomethylation correlates with the severity of the growth restriction (24). Patients with hypomethylation at ICR1 have a lower BMI than those without (BMI SDS -2.5 vs. -1.6, $p<0.05$) and more likely to have a BMI SDS ≤-2 (76% vs. 36%, p=0.01)(24). However, in children with matUPD7 postnatal reduction in height SDS is more likely (133) and height ≤-2SDS at examination was found more frequently than those with H19/IGF2 LOM (21).

Further to the observations by both Lai et al (Lai et al 1994) and Price et al (20) that asymmetry was commonly associated with the classical facial features, patients with H19/IGF2 LOM are also more likely to have asymmetry than those without (76% vs. 43%, p=0.04) (24). Bartholdi et al found asymmetry in 65% of those with hypomethylation, 20% of those with matUPD7 and 40% of the idiopathic cases (34). These findings were supported by Dias et al who found that SRS patient with H19/IGF2 LOM were more likely (81%) to have asymmetry than those with matUPD7 (10%, p=0.007) or no abnormality found (20%, $p<0.001$) (35). Clinodactyly is one of the classical features described in SRS and is found more commonly in H19/IGF2 LOM (75% vs. 45%, p=0.03) (21).

Cases with H19/IGF2 LOM may have mildly but not significantly higher OFC than those with matUPD7 (OFC SDS -0.48 and -0.95 respectively) (35). However, Netchine et al. found that cases with loss of methylation were more likely than those without to have relative macrocephaly at birth (96% vs. 64%, p=0.02) and a prominent forehead (100% vs. 79%, p=0.04) (24). Triangular facies and low-set or posteriorly rotated ears are described more commonly in matUPD7 (21).

In early reports of SRS, motor delay was documented. However cognitive difficulties were not consistently recognised. Lai et al found a mean IQ of 85.9 which was almost 1 SD below the population mean of 100 (23). The same study found that: reading comprehension was delayed more than arithmetic performance; 36% of that cohort had a statement of educational need; 48% (n=12) had received speech therapy. These findings are supported by other authors who identified psychomotor retardation in six of nine patients studied by Kotzot et al (98) and speech delay in matUPD7 reported by Hannula et al. (132). Furthermore, a prospective study of 64 patients with SRS found that comparing matUPD7 with H19/IGF2 LOM cases, global developmental delay was more common in matUPD7 (65% vs. 20%, p=0.001) and that speech therapy was more often received (67% vs. 32%, p=0.03) (21).

Other congenital anomalies are reported less frequently in matUPD7 cases (98) and major congenital anomalies are found more frequently in H19/IGF2 LOM (21). Myoclonus-dystonia has been described in a patient with matUPD7 (97) and movement disorders were described only in matUPD7 SRS in a subsequent study (21). This is likely related to the loss of expression of ε-sarcoglycan (*SGCE*), a gene located at chromosome 7q21.3, which is imprinted in some tissues and expressed only from the paternal allele. Mutations in the gene inherited from a father cause myoclonus-dystonia and it is predicted that matUPD7 is another mechanism that can cause a loss of expression. Not all obligate carriers show symptoms and non-penetrance has been suggested as a mechanism (96). Variable expressivity would potentially explain why myoclonus is not observed in every case of matUPD7 SRS.

Improved understanding of genotype-phenotype correlations in SRS would permit disease stratification and tailoring of patient care. In order to evaluate these correlations, large cohorts are required, which is challenging in this rare condition. However, it is important to consider differences between genomic subgroups when determining the adult phenotype of SRS.

1.11 Diagnosis of SRS

1.11.1 Clinical diagnostic criteria

SRS is diagnosed clinically, however the heterogeneous and predominantly non-specific features can make this challenging. Tanner used the first diagnostic criteria: a) height 2 SD or more below the 50th centile on British standards; b) birth weight 2 SD or more below the 50th centile on Tanner-Thompson standards (adjusted for gender, gestational age, birth order and maternal height); and c) absence of any other clinical phenotype that would account for the short stature (14). Those criteria were amended to add the classical features of clinodactyly, triangular facies, and low-set ears (25).

1.11.2 Clinical scoring systems

These criteria have been adapted more latterly and to-date six scoring systems, to determine a clinical diagnosis or direct molecular testing, have been proposed (summarised in Table 1.3). Those of Lai et al. (23) and Price et al. (20) were published before H19/IGF2 LOM had been described and the former before matUPD7 was reported.

In 1999 Price et al. used single-observer examination to assess a cohort of patients who were previously diagnosed with ‘definite’ or ‘likely’ SRS. 50 patients were described and then divided

into those with classical features of SRS and those with a milder facial phenotype. Those groups were then sub-classified by birth weight greater or less than -2 SDS. Of the 31 patients who had classical facial features, in the majority they met at least four of the key criteria (birth weight ≤−2 SDS; poor postnatal growth i.e. ≤−2 SDS at diagnosis; preservation of OFC; classical facial phenotype; and asymmetry), and importantly matUPD7 cases were not found in this group (20).

Netchine et al. (2007) proposed that SGA birth weight was compulsory for the diagnosis and included feeding difficulties during early childhood for the first time. This was defined when reduced food intake for age had been documented, where lack of appetite necessitated multiple meals of excessive duration, or when enteral feeding was required. The study (n=58) demonstrated a molecular abnormality in 69% of cases meeting the proposed diagnostic criteria (24). The use of SGA as a mandatory criterion for the diagnosis of SRS could increase the likelihood of detecting a molecular cause but might result in milder cases of SRS not being detected.

Bartholdi et al. (2009) proposed a scoring system to assess SRS severity and guide molecular testing. Importantly, for the adult patients included (approximately 5% of the cohort), facial features and body asymmetry were assessed from medical records or childhood photographs. Amongst other information noted by the authors to be important diagnostic features of SRS, feeding difficulties were omitted from the data analysis owing to imprecise or missing reports. Scoring was performed by an author who had not examined the patients and was blinded to the molecular results (see Table 1.3 for scoring system). Of the patients with suspected SRS and adequate clinical details, 63% (106/168) scored ≥8 points and were classified as having SRS. Of these patients, 39% were found to have an epimutation at ICR1 but none were found to have any abnormality at ICR2 and 98% (41/42) with confirmed SRS were diagnosed using the scoring system. 70% (7/10 patients) with matUPD7 met the diagnostic criteria for SRS. The authors suggested testing first for 11p15 epimutations in patients with higher (10-15) scores and for matUPD7 in patients with lower (8-9) scores (34). The association between a higher score and epimutations is consistent with (epi)genotype-phenotype correlations subsequently described (21), owing to the weighting given to asymmetry in this study.

Table 1.3 Summary of published clinical scoring systems for Silver-Russell syndrome. SRS = Silver-Russell syndrome, SD = standard deviation, SDS = standard deviation score, BW = birth weight, BL = birth length, OFC = occipito-frontal circumference, OFD = occipito-frontal diameter, * e.g. Small chin, thin lips, down-turned corners of the mouth, late closure of fontanelle. § e.g. brachymesophalangy, syndactyly of toes, inguinal hernia, pigmentary changes. Mid-parental target height calculated as: ((father's height + mother's height)/2) + 6.5 cm for boys and - 6.5cm for girls. ⅄ Defined as a forehead that projects beyond the facial plane when viewed from the side. ✝ Defined as leg length discrepancy of ≥ 0.5 cm or arm asymmetry, or LLD <0.5 cm with at least two other asymmetrical body parts – one being a non-face part. ✠Use of a feeding tube or cyproheptadine for appetite stimulation.

	Lai et al. 1994 (23)	**Price et al. 1999 (20)**	**Netchine et al. 2007 (24)**	**Bartholdi et al. 2009 (34) "SRS severity scoring system"**	**Dias et al. 2013 (35) "Birmingham SRS screening score"**	**Azzi et al. 2015 (32) "Netchine-Harbison clinical scoring system"**
Birth parameters	BW ≤-2 SD below population mean	BW ≤-2 SD below mean	BW and/or BL ≤-2 SDS	Weight ≤10th centile Length ≤10th centile	BW SDS <-2	BW and/or BL ≤-2 SDS
Growth pattern	Height for age at diagnosis ≤-2 SD below population mean	Growth ≤-2 SD below mean at diagnosis	Height ≤-2 SDS at age 2 years or nearest follow-up	Height ≤3rd centile OFD ≥3rd and ≤97th centiles Normal cognitive development	Height SDS <-2 after 2 years of age	Height at 24 ± 1 months ≤-2 SDS or height ≤-2 SDS from mid-parental target height
Craniofacial features	Characteristic craniofacial shape (as described by Russell)	Preservation of OFC	Relative macrocephaly at birth; OFC SDS ≥1.5 above BW and/or BL SDS	Relative macrocephaly at birth; OFD SDS ≥1.5 above BW/BL SDS	Relative macrocephaly; head circumference SDS >1.5 than height SDS)	Relative macrocephaly at birth; head circumference SDS birth ≥1.5 above BW and/or BL SDS
		Classical facial phenotype	Prominent forehead during early childhood	Triangular shaped face High/bossing forehead Other *		Protruding forehead as a toddler ⅄

	Lai et al. 1994 (23)	**Price et al. 1999 (20)**	**Netchine et al. 2007 (24)**	**Bartholdi et al. 2009 (34)** **"SRS severity scoring system"**	**Dias et al. 2013 (35)** **"Birmingham SRS screening score"**	**Azzi et al. 2015 (32)** **"Netchine-Harbison clinical scoring system"**
Asymmetry	Asymmetry of limbs body/face	Asymmetry	Body asymmetry	Asymmetry of face/body/limbs	Asymmetry of limbs/body	Body asymmetry †
Other features	Clinodactyly		Feeding difficulties during early childhood	5th finger clinodactyly Genital abnormalities Other §		Feeding difficulties ✠ and/or low BMI (BMI ≤-2 SDS) at 24 months
Criteria required to meet positive diagnosis	Three required	Authors state 'the major features [listed above]…form useful criteria when considering a diagnosis of SRS'	Mandatory feature BW/BL plus three from the remaining criteria	Presence of each item scores 1 apart from asymmetry (scored 0 if absent; 3 if present). Scores ≥8 classified as SRS.	Three required– to warrant molecular testing	Scores ≥4 'likely SRS' Scores 0-3 'unlikely SRS'
Patients' age range	Included patients only aged 6-12 years (mean 8.75)	Ages 0.84 – 35.01 years	Ages not reported, however focuses on early childhood	Age range 1-42 years (mean 6.5)	0.25-21 years (median 3.25)	1.05-20.06 years (mean 6.61)

Dias et al. (2013) modified diagnostic criteria to guide molecular genetic testing by non-specialist clinicians and reduce the number of subjective elements included. The authors reported a sensitivity of 82% (78% below 2 years old) and a specificity of 80% (75% below 2 years old) where sensitivity referred to the ability of the scoring system to identify cases which then had a positive genetic result; and specificity the ability of the scoring system to exclude the diagnosis in cases which then had negative results on genetic testing. There is limitation to the interpretation of these results in view of the widely accepted finding, which was indeed reported by the authors, that the (epi)genetic cause is unknown in up to 50% of SRS cases (35). A high specificity would likely mean that patients with compatible clinical features of SRS with currently unidentified epi-/genetic causes would not be detected therefore the application of this scoring system is restricted to and by current knowledge.

The 'Netchine-Harbison clinical scoring system' (NHCSS) was proposed in 2015 and modifies the scoring system proposed by Netchine et al in 2007 ((32)). Sixty-nine participants with SRS who attended a MAGIC foundation (SRS/SGA lay support group) meeting in the USA, were examined by two paediatric endocrinologists, who together assessed the following clinical features: birth weight and SDS; head circumference and SDS; postnatal growth; feeding history or body mass index (BMI) at 2 years; forehead appearance; body asymmetry. The following features were reviewed in addition: downturned mouth; clinodactyly of the 5th finger; shoulder dimples; 2/3 syndactyly of the toes; low muscle mass; prominent heel; autism/pervasive developmental disorder; diagnosed cognitive disabilities. Both evaluators (Netchine and Harbison) are highly experienced in the diagnosis and management of patients with SRS. Where participants were aged above three years, the forehead appearance was assessed on photographs taken between one and three years of age. The patients were first classified by the proposed criteria (see Table 1.3). 60 of 69 patients met ≥4 of the criteria and were classified as 'likely SRS [SRS]' and of these patients, molecular abnormalities were detected in 76.7% (46/60). Nine patients met ≤3 criteria and were classified as 'unlikely-SRS [SRS]'. Overall, the NHCSS successfully detected 97.9% of the patients with known molecular abnormalities. This sensitivity of (97.9%) is preferable to the Birmingham (83.7%) (35) and Netchine et al (91.5%) (24) scoring systems (32). Thus, the recent international consensus on the diagnosis and management of SRS recommended the use of the NHCSS (15).

The scoring systems show considerable overlap in the definitions of poor prenatal and postnatal growth, and key features in the phenotype, however feeding difficulties have now been emphasised. Where the participants' ages were reported, the scoring systems included both

children and adults, apart from one. However, the mean and median ages suggest there is a right skew in the age distribution (i.e. there are more cases with younger ages and fewer cases with older ages) and childhood features are emphasised in the four most recent publications. No clinical scoring system for adults with SRS has been published.

1.12 Differential diagnoses of SRS

The differential diagnoses considered in this section have been included because they have clinical features in common with SRS. These features comprise some but not all of the following: pre- and post-natal growth failure, feeding difficulties, asymmetry, hypotonia, delay in motor development, early puberty, delayed bone age, facial features and café-au-lait macules.

In addition, where molecular testing of clinically-diagnosed SRS cohorts has excluded H19/IGF2 LOM and matUPD7, additional molecular genetic testing has occasionally identified individuals with the differential diagnoses below.

1.12.1 Temple syndrome

Temple syndrome is due to aberrant expression of imprinted genes on chromosome 14 at 14q32 and can be due to maternal UPD14, a paternal deletion at 14q32 or an epimutation at the 14q32 intergenic (IG) DMR (134). Patients with Temple syndrome, present with low birth weight and frequently have feeding problems in the neonatal period and may fulfil the criteria of SRS although asymmetry is less commonly observed. Hypotonia with delayed motor milestones occur more commonly, often with diminished IQ compared to SRS. Small hands and feet, and early puberty, more marked than SRS, are found in the majority. The typical facial appearance differs from SRS with a broad forehead but no triangular facial shape and a fleshy nasal tip. Obesity is common in adulthood and is particularly truncal (134). Temple syndrome is more difficult to distinguish from SRS in adulthood as final height is similar and the facial features of SRS are less apparent after childhood. One epimutation compatible with Temple syndrome was detected in 60 patients who met the diagnostic SRS criteria using the NHCSS (32). Furthermore, two cases of epimutations were found in 85 Japanese patients who met the 2007 Netchine et al diagnostic SRS criteria and were negative for H19/IGF2 LOM and matUPD7 (135). Testing for matUPD14 and copy number or epimutation at 14q32 should be considered in clinically diagnosed SRS patients who are negative on standard molecular tests.

1.12.2 IMAGe syndrome

The main clinical features of IMAGe syndrome are IUGR, metaphyseal dysplasia, adrenal hypoplasia congenita, and genito-urinary abnormalities (in affected males) (136). The pattern of growth restriction results in birth weight SDS -2 to -4 and birth length SDS -1.8 to -4.5 with OFC SDS -2 to -3. Poor postnatal growth, with height SDS -2.7 to -6.5 and weight SDS -2 to -7, is a consistent finding. Of six patients where OFC was reported, these were normal in four and -4 SDS in two (137). Growth hormone deficiency has been reported in one case (138).

Skeletal findings were reported in all cases with delayed bone age with short stature being the most commonly reported. These features overlap with the SRS phenotype, however the contrasting features of metaphyseal and epiphyseal dysplasia of the long bones have also been reported and are evident in most cases by five years of age (however there are relatively few reports of skeletal surveys in classical SRS at this age). Adrenal insufficiency has been reported in all cases and adrenal crisis described within the first week to month in most cases (137). The eldest patient to be diagnosed was five years old at the time and had milder adrenal insufficiency (139). Genitourinary anomalies were reported in nearly all males with IMAGe syndrome and no males are reported to have procreated. The typical facial features of frontal bossing, low-set or small ears, and a flat or broad nasal bridge have been described as 'similar to the triangular facies seen in Silver-Russell syndrome' with cleft palate and uvula sometimes reported. Similarly to SRS, hypotonia has been described and may be associated with delayed motor milestones however, developmental outcome is generally believed to be normal (137).

There are no clinical diagnostic scoring systems used to differentiate cases from SRS. Of 20 patients with descriptions of the findings at presentation, 18 patients were diagnosed antenatally or during the neonatal period (137). Gain of function mutations in *CDKN1C* have been reported as causative when detected on the maternally expressed allele (110). This gene lies within the chromosome 11p15 imprinting cluster. As described in section 1.10.6, a family with a gain-of-function mutation in *CDKN1C* has been reported in familial SRS (111). This disorder may be very difficult to differentiate from SRS until more cases are reported. However, the presence of skeletal abnormalities and adrenal insufficiency in all cases of IMAGe syndrome and the absence of the feeding difficulties seen in SRS are distinguishing features.

1.12.3 Maternal uniparental disomy for chromosome 20

The first human report of maternal uniparental disomy for chromosome 20 (matUPD20) was a case of growth retardation (height and OFC both <3rd centile) with a history of IUGR at 30 weeks'

gestation. There were mild dysmorphic features; short philtrum, thin upper lip, high palate, prominent supra-orbital region, large and posteriorly rotated ears, hyperextensible joints and syndactyly of the 2nd to 5th fingers. Motor development and intelligence were normal, however hyperactivity was present (140). Another case of matUPD20 was described after screening of 21 patients with IUGR (141). The clinical description of this patient included: 'insufficiency of the placenta' during pregnancy, birth weight <3rd centile, birth length <10th centile, macrocephaly (no measurement or centile given), and bilateral clinodactyly. The patient additionally had strabismus and hyperactivity. Apparent matUPD20 has also been detected in a cohort of individuals with clinical features suggestive of SRS; 1 of 127 cases (142) and 1 of 69 cases (32). In this last case, maternalisation of imprinted loci on chromosome 20 was detected by methylation analysis, however confirmation by microsatellite analysis was not possible because parental samples were unavailable.

Overlapping clinical features of growth failure and feeding difficulties have been described in SRS and matUPD20. However, in eight patients with matUPD20 there were no shared clinical features in addition to the growth phenotype, the OFC centiles were in proportion in all apart from one case, there were no overt dysmorphic facial features or body asymmetry (85). Therefore, the characteristic, childhood facial shape, relative macrocephaly and presence of body asymmetry distinguish classic SRS from matUPD20.

1.12.4 The 3-M syndrome

3M syndrome is heterogeneous and due to mutations in *CUL7*, *OBSL1* and *CCDC8.* It is an autosomal recessively inherited condition characterised by short stature, somewhat distinctive facial features, and skeletal abnormalities (143). Intra-uterine growth retardation, relative head sparing, and short fifth fingers are also described and it was recognised as a differential diagnosis for SRS as early as 1987 (18). Feeding problems are commonly reported (144). The facial features include: triangular facial shape with a pointed chin, anteverted nares, and fleshy lips. Patients have been described to have prominent heels with some radiographic features including slender long bones and tall vertebral bodies (145). Hypospadias and male hypogonadism have also been described (146). Other features, which distinguish from SRS include: a short, broad neck, deformed sternum, short thorax, square shoulders, winged scapulae, and hyper-lordosis (146) and absence of asymmetry (144). The growth failure in 3M is more severe than in SRS (147). A good response to growth hormone treatment has been reported in some cases (147), however another report describes a child in whom there was a poor response to early initiation of GH treatment despite evidence of GH deficiency. This child reached a final height SDS of -6.42 (144).

1.12.5 Mosaic trisomy 22

Mosaic trisomy 22 is a rare condition, however, unlike non-mosaic trisomy 22, it is compatible with prolonged survival. Asymmetry and poor growth are the main reasons why SRS is considered within the differential diagnosis. Crowe et al. reviewed cases of both and described overlapping clinical features – small for gestational age, microcephaly, epicanthic folds, micrognathia, low-set and malformed ears, pre-auricular pits and tags, webbed or short neck, congenital heart defects, hypoplastic nails, and fifth finger clinodactyly. The authors found that specific features of the mosaic phenotype included: 'failure to thrive', ptosis, low posterior hair line, mental retardation, syndactyly, dental anomalies, hearing loss, ovarian failure, hemi-atrophy, and streaked pigmentation (148). The majority of patients have abnormal cognitive development and this is often a useful discriminating feature that leads to consideration of this diagnosis. However, the mosaic nature of the condition means that cognitive development is variable.

1.12.6 12q14 microdeletion syndrome

The key features of 12q14 microdeletion syndrome are severe prenatal and postnatal growth failure, short stature, osteopoikilosis and mental retardation (149). The diagnosis of SRS was considered in one of the first three cases described. Mari et al (150) described a patient with severe pre- and postnatal growth failure (birth weight <10th centile with length SDS -4 and at 18 months weight SDS -4.9 and length SDS -5.3) without osteopoikilosis. The clinical features also included developmental delay, hypoglycaemia, poor feeding and a prolonged requirement for nasogastric tube feeding. Similar facial features to those seen in SRS were described and the diagnosis was considered clinically. These were triangular facies with a prominent forehead, down-turned corners of the mouth, a high palate, and slight micrognathia. However, some features reported were not associated with SRS, such as the absence of relative macrocephaly (OFC SDS -3.66 and -5.6 at birth and 18 months respectively), hypocalcaemia and ventriculoseptal defect (150).

Amongst a cohort of 20 patients with a clinical diagnosis of SRS who were negative for matUPD7 and epimutations at chromosome 11p15, one was found to have a 12q14 microdeletion (151). The positive diagnosis of SRS was based on birth weight or length below the third percentile, lack of postnatal catch-up growth, and at least two of the following: typical face, relative macrocephaly and asymmetry. The patient had IUGR (noted in 20th week of gestation), was born at term with birth length SDS -2.59 and weight SDS -1.83. Feeding difficulties and a 'squeaky' voice were reported. There was evidence of postnatal growth failure with height, weight and OFC SDS scores of -4.5, -5.4 and -3.3 respectively when assessed at age 1 year and 9 months. The

patient displayed other features of SRS; a prominent forehead, mildly triangular face, slightly dysplastic ears and clinodactyly of the fifth fingers, but no asymmetry was present (151). The microcephaly and degree of cognitive deficit assist in differentiation from SRS.

1.12.7 Mulibrey Nanism

*Mu*scle-*li*ver-*br*ain-*ey*e nanism was described by Perheentupa et al (152) and involves prenatal and postnatal poor growth and cardiac abnormalities. The characteristic facial features overlap with SRS, including a triangular-shaped face with prominent forehead and there is often muscular hypotonia. However, there are contrasting features of: a J-shaped sella turcica (although this requires imaging which is often not done in SRS) and characteristic retinal changes described as yellow spots. The liver is often enlarged. The eye features are the key distinguishing findings when present and they can also include chorioretinitis and retinitis pigmentosa. The common cardiac features are pericarditis and heart failure as the disease progresses. The majority (80%) of patients are Finnish (153) and this autosomal recessive disorder is due to mutations in *TRIM37*.

1.12.8 Chromosome breakage syndromes

The chromosome breakage syndromes include Fanconi anaemia (also known as Fanconi pancytopaenia), Bloom syndrome, and Nijmegen breakage syndrome. These conditions are inherited in an autosomal recessive manner and cause chromosome fragility. Bloom syndrome is most common in Ashkenazi Jews whereas Nijmegen breakage syndrome is particularly prevalent in Poland.

Fanconi anaemia causes bone marrow failure and aplastic anaemia. The following are common features: short stature (63%), café-au-lait macules (64%) and congenital anomalies, particularly skeletal (71%). Skeletal manifestations include scoliosis, absent thumbs, radial anomalies, renal and urinary tract (34%) as well as male genital abnormalities (20%). Mental retardation (16%) is reported (154). Differentiating factors from SRS are: 1) the reduced age of survival in Fanconi anaemia with a reported median of 16-23 years (154) (however this is not a useful feature in younger people) and 2) microcephaly and microphthalmia (155). Spontaneous chromosome breakage should be examined in mitomycin C-treated cultures and is a screening test prior to gene sequencing. Gene sequencing is challenging because this is a heterogeneous condition with several genes (*FANC A-L*) causing similar symptoms.

Characteristics of Bloom syndrome that are also found in SRS are: small for gestational age birth weight, short stature, reduced subcutaneous fat, high-pitched voice; small jaw; and café-au-lait macules. However, the distinctive features include: microcephaly, a long narrow face, prominent

nose, oversized ears, hands and feet, long limbs; normal muscle development; and low immunoglobulins with resultant susceptibility to infection (156). There is usually a characteristic telangiectatic erythema following sunlight exposure although this photosensitive rash may not develop until after puberty. A recent report described two patients who were initially diagnosed with SRS and treated with GH (157). Bloom syndrome should be particularly considered in patients with skin abnormalities or where there is consanguinity. Furthermore, IGF1 levels on GH should be monitored: in the two patients with Bloom syndrome who were originally diagnosed with SRS, IGF1 levels increased to >3.5 SDS on treatment. IGF-BP3 levels remained normal (157). Bloom syndrome is due to mutations in *RECQL1* (RecQ protein-like type 1).

Growth failure in Nijmegen breakage syndrome may be pre- or postnatal, café-au-lait macules are common (50%), and delayed bone age has been reported (158). These features, and retrognathia, resemble SRS, although distinctive characteristic facial features are: a receding forehead; prominent mid-face with long nose; long philtrum; upward slanting palpebral fissures; large dysplastic ears with freckles and scleral telangiectasia (158). In contrast to SRS, microcephaly is commonly reported (OFC <3rd centile in 75%) and is frequently progressive. The severity of microcephaly is marked and has been reported as low as -9 SDS (159). Despite this severe microcephaly, developmental motor milestone are usually attained, in contrast to motor delay which may be present in SRS. Cognitive development is initially normal or borderline, however in older children mild to moderate intellectual disability is reported (159) and this also distinguishes the condition from SRS. Most cases are due to mutations in *NBS1*

Patients with Fanconi anaemia, Bloom syndrome and Nijmegen breakage syndrome all have increased predisposition to malignancies. Therefore, they are particularly important differential diagnoses because, in contrast to SRS, GH treatment is contra-indicated.

1.12.9 Floating Harbor syndrome

Short stature with markedly delayed bone age, distinctive facial appearance, delayed expressive language are key features of Floating Harbor syndrome. The characteristic features of triangular facial shape with a narrow nasal bridge broadening at the tip are similar to SRS, although micrognathia appears more marked in SRS. Other overlapping findings include gastro-intestinal motility problems (reflux, constipation and coeliac disease), cryptorchidism, renal anomalies, markedly delayed bone age and a high-pitched or nasal voice (160). Further distinguishing features that are not typical of SRS include severe speech delay, deep set eyes, a low-hanging columella, large nares, brachydactyly, and broad fingertips and first toes. Prenatal growth is less affected in Floating Harbor syndrome than SRS with one report demonstrating only 27% with

birth weight below the 3rd centile (161). Although the facial gestalt of Floating Harbor syndrome with some similarities to Rubinstein-Taybi syndrome distinguishes this from SRS, this can be difficult. Truncating mutations in *SRCAP* have been found to be causative for Floating Harbor syndrome. SRCAP is an SNF2-related chromatin-remodelling factor which acts as a co-activator for CREB-binding protein, which is the major cause of Rubenstein-Taybi syndrome therefore this association explains the clinical similarities.

1.12.10 An approach to testing to exclude these disorders

As discussed above, there are several disorders with clinical features that overlap SRS. It is important to consider the differential diagnoses as treatment (and response to treatment) may depend on the diagnosis. Alternative diagnoses are frequently associated with different inheritance patterns which would affect genetic counselling for patients and their relatives.

If a patient fulfils the criteria for SRS but standard SRS testing on chromosomes 11 and 7 is negative, it is important to consider:

1. Karyotype to exclude a copy number disorder in blood and skin
2. Epigenetic testing of chromosomes 14, 16 and 20
3. Chromosome breakage testing particularly where microcephaly is present
4. Skeletal survey
5. Clinical exome to exclude *CDKN1C* mutations, *IGF2*, genes within the IGF2 pathway and genes for the differential diagnoses discussed above.

The international consensus on the diagnosis and management of SRS proposed a flow chart for the investigation and diagnosis of SRS, which includes the use of the NHCSS, highlights the presence of relative microcephaly as a discriminating factor to encourage consideration of differential diagnoses, proposes additional molecular testing (14q32 analysis, matUPD16, matUPD20, *CDKN1C* mutation analysis, *IGF2* mutation analysis) and advocates alternative tissue analysis (15).

For patients in whom the diagnosis of SRS is confirmed, either clinically or molecularly, treatment to improve growth is available. In addition to improving nutritional status, one of the key treatments to consider in SRS is growth hormone treatment.

1.13 Growth hormone treatment in SRS

1.13.1 Growth hormone and the growth hormone IGF1 axis

Human growth hormone (GH) is a 191-amino-acid, single-chain, polypeptide, which is secreted by somatotroph cells within the anterior pituitary gland. Hypothalamic hormones control the release of GH: GH-releasing hormone is stimulatory and somatostatin is inhibitory and their interaction results in the release of GH in a pulsatile pattern, which then circulates in blood bound to binding proteins (Figure 1.6).

GH acts on the liver, adipose tissue, muscle and bone via the growth hormone receptor. The indirect actions are mediated by IGF1 and these predominate. IGF1 is a peptide comprised of 70 amino acids, which is produced in the liver as a result of GH binding to its receptor. Therefore, the concentration of IGF1 in the blood is closely linked to secretion of GH. Most IGF1 is present in the circulation bound to IGF-binding proteins (IGFBPs), which transport and prolong the half-life of IGF1. The predominant binding protein is IGFBP3, which is responsible for approximately 90% of insulin-like growth factor binding capacity (162), and is able to modify its action. The IGF1 ternary complex is composed of IGF1, IGFBP3 and acid-labile subunit (ALS).

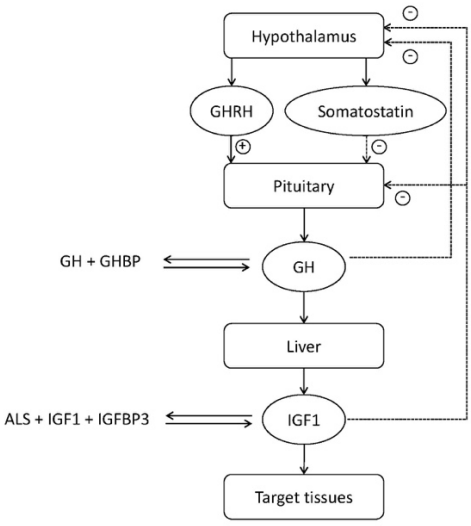


Figure 1.6 A schematic of the growth hormone-insulin-like growth factor axis. GHRH = growth hormone releasing hormone, GH = growth hormone, GHBP = growth hormone binding protein, ALS = acid-labile subunit, IGF1 = insulin-like growth factor 1, IGFBP3 = insulin-like growth factor binding protein 3. + indicates a stimulatory process and - indicates an inhibitory process. GH also acts directly on adipose tissue, muscle and bone.

IGF2 is predominantly a fetal growth factor in humans and its postnatal role is poorly understood (163). The effects of IGF2 are mediated via the IGF1 and IGF2 receptors. It is thought to act in a paracrine and autocrine fashion therefore serum levels may not reflect its action (164).

Normal serum IGF2 levels have been demonstrated in 20 clinically diagnosed SRS patients aged 3.1-12.4 years (mean 5.4) and IGF2 levels were higher, although with a wider variation, in SRS than age-matched controls with SGA (165). The same study showed that three out of four children with loss of methylation at *IGF2* had serum IGF2 levels at the upper end of the normal range. Postnatal serum IGF2 levels have been reported as normal (normal range -2 to2 SDS) in the majority of cases of clinically diagnosed and molecularly confirmed SRS (24). Of the 17 patients, in that study, with *IGF2* loss of methylation, one had serum IGF2 levels above the normal range and two had levels below. This is thought to be explained by postnatal IGF2 being produced in the liver by a non-imprinted promoter meaning that postnatal levels are not representative of the in-utero environment (Netchine personal communication).

Two patients with SRS have been reported with low *IGF2* expression in cultured skin fibroblasts (116): in leukocyte DNA one patient was demonstrated to have H19/IGF2 LOM however the other had normal methylation levels. The difficulty in obtaining tissues other than blood may explain the paucity of evidence on *IGF2* expression.

1.13.2 Growth hormone treatment for improving linear growth and final height

Recombinant GH has been available since 1985 and although administration was initially via intramuscular injection, the subcutaneous route is now preferred. It remains an expensive treatment with an estimated cost of £5000 per patient year.

GH is presently licensed in the UK for the following conditions (percentage of patients indicated in brackets): GH deficiency 57.4%, Turner syndrome 18.7%, SGA 5.2%, Prader-Willi syndrome 4.6%, chronic kidney disease 2.5%, unlicensed indications 11.6% (166). No data were available for short stature homeobox (SHOX) deficiency, which received a licence in 2006.

The different conditions for which GH is prescribed include GH-deficient and non-GH-deficient diseases. In GH deficiency, the purpose of therapy is growth hormone replacement. In the non-GH-deficient conditions that are associated with short stature, the treatment aim is to normalise short and long-term growth – therefore height velocity as well as final adult height. In

conditions where GH secretion is either normal or there is resistance, doses are usually higher (166) (see Table 2).

Table 1.4 Growth hormone doses for the UK paediatric licence (166).

Diagnosis	Doses	
	µg/kg/day	**mg/m²/day**
GH deficiency	23-39	0.7-1.0
Turner syndrome	45-50	1.4
Chronic kidney disease	45-50	1.4
Prader-Willi syndrome	35	1.0
Small for gestational age	35	1.0
SHOX deficiency	45-50	1.4

1.13.2.1 Growth hormone treatment for children with short stature who were born small for gestational age

GH is prescribed in patients with SRS under the licence for SGA, which was granted in the UK in 2003. This licence stipulates that eligible children were born with a weight and/or length <-2 SDS, fail to catch-up in their growth (height velocity SDS <0 during the previous year) by age 4 years or later, and are short both compared to their parents (parental-adjusted height <-1 SDS) and their peers (height <-2.5 SDS). The licence in the USA for GH treatment in SGA is approved for patients who do not catch up in growth by age 2-3 years (167).

GH treatment has been demonstrated to improve growth rate in children born SGA (168, 169) but without improvement in height-for-bone-age (168). These groups included, but were not limited to, children with SRS. GH treatment was subsequently confirmed to cause a dose-dependent increase in height velocity and weight gain in patients born SGA (170-172) as well as height SDS (173). Increased response has been associated with younger age at start of treatment and shorter stature (170) and in contrast, also with taller height at start of treatment and increased treatment duration (174). The dose-dependent relationship with GH hormone treatment was maintained when near-adult height was examined (170). Increased height velocity does not continue after cessation of GH (175). Increased adult height SDS has been reported in GH-treated children with SGA (176) and quantified in a randomised trial as a 4 cm increase in final height

(177). A systematic review including six randomised controlled trials in SGA confirmed that adult height is approximately 4 cm greater with GH treatment (178).

1.13.2.2 Growth hormone treatment in SRS

The prevalence of GH deficiency in SRS is between 0.02% (1 in cohort of 50 clinical cases) (20) and 22% (4 of 18 in a group of children with a clinical diagnosis) (23). There have been few studies of GH treatment specifically in SRS and randomised controlled trials would be difficult since it would be unethical to withhold licensed treatment for a condition and administration of placebo would involve daily injections. Swedish data has shown that a subset of SRS patients received GH at a younger age with a higher starting dose than children born SGA (174). The reason for this is unclear but may reflect individual clinical practice.

Growth hormone treatment was first prescribed in SRS in 1971 (179). Lai et al (1994) found that patients clinically diagnosed with SRS being treated with GH had a mean height SDS of -1.84 compared to -2.82 in those untreated and weight SDS of -1.16 and -1.52 respectively (23). In clinically diagnosed SRS, treatment with GH has been demonstrated to increase height velocity and height SDS over 5 years of treatment (180). The final height data of 26 clinically diagnosed SRS patients treated for a median of 9.8 years (range 7.0-15.7) showed an increase in height SDS of 1.4 (from -2.74 (range -5.22-2.20) to -1.33 (-3.5-1.01)). The majority (23/26) of patients received a GH dose of 10 mg/m^2/week [1.43 mg/m^2/day] which is higher than the current recommended dose for short stature and SGA (47). Height at start of treatment was inversely associated with height gain although height at start of puberty was positively related to height gain and age at start of treatment was not found to affect height gain (47).

In mixed cohort of clinically diagnosed and molecularly confirmed SRS, treatment for a mean of 5.6 years with GH at a dose of 51 ± 13 mcg/kg/day has been reported to cause height SDS increments, from baseline to adult height, of 1.18 in males (baseline SDS -3.02 ± 1.06) and 1.47 in females (baseline SDS -3.96 ± 1.22). These compared to untreated males and females where height SDS increments were found to be 0.36 and -0.05 respectively. Matched patient pairs showed that treated patients were taller by 1.5 ± 0.82 SDS (11.1 ± 6.1 cm) in males and 0.7 ± 2.22 SDS (4.0±12.7 cm) in females. However, a limitation to the interpretation of these results is the use of gonadotrophin releasing hormone (GnRH) analogues to delay pubertal onset in 16 of the 37 patients who were treated with GH (46). GH treatment in seven patients with clinically diagnosed SRS and lower limb asymmetry was not associated with significant alteration in asymmetry (181).

A study evaluating GH treatment in 62 cases of SRS (including 42 molecularly confirmed) showed that height SDS was lower in SRS compared to non-SRS SGA and short stature; -3.67 (SD 1.0) and -2.92 (SD 0.6) respectively ($p<0.001$). Total height gain SDS from start of treatment until adult height was similar in SRS and and non-SRS; 1.30 (SD 1.0) and 1.26 (SD 0.8) respectively ($p=0.081$). Adult height SDS was lower in SRS than SS SGA: -2.17 (SD 0.8) vs -1.65 (SD 0.8) in non-SRS respectively ($p=0.051$). The GH dosage was 1 mg/m^2/day in all cases (182).

This studies presented in this thesis will evaluate adult height in SRS in individuals treated and untreated with GH and follows on from previous studies (46, 182). The aetiology of the greater height gain in males compared to females demonstrated in one previous study is unknown (46). The outcome of the study presented in this thesis might support or contradict this finding and suggest a new aspect for research in SRS.

Table 1.5 Summary table of the effects of GH treatment on height in SRS.

Study	**GH-treated**	**GH-untreated**	**GH dosage**
Lai et al (23)	Mean height SDS -1.84	Mean height SDS -2.82	
Toumba et al (47)	Height SDS increment of 1.44		1.43 mg/m^2/day
Binder et al (46)	Height SDS increment of 1.18 in males and 1.47 in females		51 mcg/kg/day
Smeets et al (182)	Height SDS increment of 1.30		1 mg/m^2/day

1.13.3 Additional effects of growth hormone treatment in Silver-Russell syndrome and children with short stature who were born small for gestational age

1.13.3.1 Body composition

In addition to the effects on height velocity, GH treatment has been shown to reduce fat mass, increase lean body mass over 6 to 12 months in children with short stature (183). In children with short stature who were born IUGR (18/25 had features of SRS), GH treatment has been shown to reduce triceps and subscapular skinfold SDS during the first year of treatment, in a dose-dependent manner, although an increase was found over the subsequent four years of treatment (184). Biceps, triceps, subscapular and supra-iliac skinfold thicknesses were assessed during GH treatment in 79 children with short stature and SGA, including some who had SRS. Skinfold thickness SDS were found to: 1) be significantly below zero before treatment; 2) reduce significantly during the first year of treatment; 3) then increase significantly over the next five years of treatment to values not significantly different to those before treatment (185). More recently, in short children born SGA treated with GH, reduction in limb skinfolds compared to truncal skinfolds has been demonstrated without an overall gain or loss of fat mass, however accompanying increase in lean mass was reported (172). Body composition in patients with short stature born SGA has been assessed by dual energy x-ray absorptiometry (DXA) and fat mass was not significantly lower than normal and GH treatment resulted in increased lean body mass during the first year which returned to baseline during the subsequent two years of treatment (64). A contrasting study, using peripheral quantitative computed tomography has reported reduction in fat area along with increased muscle area and improved grip strength in short children born SGA (including 7/34 with clinical SRS) after 2 years GH treatment (65).

BMI SDS has been shown to increase (from mean BMI SDS of -1.0 to -0.2) over six years of GH treatment in children with SGA (186) and a mixed group of SGA and clinically diagnosed SRS (65, 185). Furthermore, both BMI and weight have been reported to increase during GH treatment in clinically diagnosed SRS cases (weight SDS increased from -3.3 to -1.3; BMI SDS from -1.6 to -0.3), however triceps and subscapular skinfold thicknesses measured at the start and at the end of treatment were unchanged in this group (47).

In adolescents born SGA and treated with GH, six months after treatment cessation significant increases in percentage fat, fat mass and decrease in lean body mass (all were SDS values corrected for height and gender) have been demonstrated. After six months off GH, this group were similar to a group of adolescents who were born with appropriate weight for gestational age (AGA) and matched for gestation and gender, however the AGA group was older (19.2 years (SD

1.3) vs. 16.5 years (SD 0.9) (187). Fat mass, fat distribution and lean body mass in adults born SGA previously treated with growth hormone (GH) are comparable to untreated SGA adults (66) therefore the effects of GH do not appear to affect long-term body composition in adults born SGA. The studies presented in this thesis will investigate body composition.

In the study of Smeets et al (2017), which included 29 individuals with SRS and compared them to non-SRS SGA cases, the authors found that: lean body mass was lower in SRS, stayed the same during GH treatment, and initially declined following GH discontinuation but stabilised after 18 months. Baseline fat mass percentage was similar in SRS compared to SGA. During GH treatment, fat mass percentage increased and then increased further six months following treatment discontinuation. However, fat mass percentage stabilised 18 months after this compared to SGA where it continued to increase (80).

Table 1.6 Summary table on the effects of GH treatment in SGA.

Study	**Body composition change**	**Duration of GH treatment**	**Subsequent body composition change**
Walker et al (183)	Reduced fat mass, increased lean body mass	6-12 months	Not reported
Albanese et al (184)	Reduced skin fold thickness SDS	12 months	Increased skin fold thickness over subsequent four years after treatment cessation
Sas et al (185)	Reduced skin fold thicknesses	12 months	Increased over the next five years after treatment cessation
De Schepper et al (172)	Reduced limb skin folds compared to truncal skin folds and increased lean mass	2 years	Not reported

Study	**Body composition change**	**Duration of GH treatment**	**Subsequent body composition change**
Hokken-Koelega et al (64)	Increased lean body mass	12 months	Returned to baseline during next two years of GH treatment
Schweizer et al (65)	Reduced fat area and increased muscle area	2 years	Not reported
Van Pareren et al (186)	Increased BMI SDS	6 years	Not reported
Toumba et al (47)	Increased weight and BMI	Median 9.8 years	
Willemsen et al (187)			Increased fat percentage, fat mass, and decreased lean body mass from GH cessation to six months afterwards
Smeets et al (80)	Lean body mass unchanged and fat mass increased		Initial decline in lean body mass and increase in fat mass over six months from GH cessation then both stabilised over next 18 months

1.13.3.2 Metabolic health and cardiovascular risk factors and growth hormone treatment

A positive association between SGA and subsequent cardiovascular risk factors (type 2 diabetes mellitus, hyperlipidaemia, hypertension) in adulthood has been demonstrated (188). Since GH acts to raise blood glucose, and relative insulin-resistance has been reported with long-term GH treatment, there has been concern that there could be an increased risk of developing type 2

diabetes mellitus. Furthermore, GH treatment may potentiate the cardiovascular risk in patients born SGA.

Normal glucose levels have been demonstrated following one course and two courses of GH treatment (separated by two to three years in the latter case) (189). However, in short stature with SGA (including an unspecified number of clinical SRS cases) increased fasting glucose concentrations during GH treatment have been reported to occur in a non-dose-dependent manner. After treatment discontinuation, these levels returned to baseline (pre-treatment). The authors also found impaired glucose tolerance in 4% (1/27) after six years of GH treatment and 10% (3/29 [stet]) in the same cohort six months later, following treatment cessation (186).

GH treatment for SGA (including sporadic clinical SRS cases) has been shown to increase fasting insulin levels after 1 and 6 years of treatment with no dose-dependent difference observed (64). In the same study, the prevalence of impaired glucose tolerance decreased from 8% at baseline to 4% after six years of treatment.

In SGA (including sporadic clinical SRS cases) glucose-stimulated insulin concentrations have been shown to rise during GH treatment. Following this, one study reported restoration to normal reference levels for age after discontinuation of treatment (64). In contrast, another study showed that glucose-stimulated insulin levels did not revert to normal after discontinuation of GH treatment (186). This may have been related to the reduced insulin sensitivity that occurs in normal puberty. There were no new cases of diabetes reported on GH treatment (64).

GH treatment has been associated with significantly reduced systolic and diastolic blood pressures in children SGA and SRS (185), and short stature with SGA (64, 186). Reduction in blood pressure is maintained six months after discontinuation of treatment (64, 186).

HbA1c levels in patients born SGA have been shown to decrease from pre-treatment levels, after six years of treatment, and then to be maintained over the subsequent six months off treatment (186). In patients with SGA after six years of GH treatment, total, LDL and HDL cholesterol levels have been shown to decrease from baseline. However, following cessation of treatment, total cholesterol remained the same although LDL and HDL cholesterol concentrations increased significantly in girls (186). Minor but not significant, improvement in lipid profile has been described with GH treatment in clinically diagnosed SRS cases without changes in HbA1c or triglyceride concentrations (47).

In the study of Smeets et al (2017), the results of the 29 individuals with SRS included showed that blood pressure was similar at the start and end of GH treatment and was stable at 2 years

following GH discontinuation. Fasting blood glucose were normal and were lower in SRS than non-SRS SGA. Although fasting blood glucose concentration increased on GH treatment and remained high after two years, type 2 diabetes mellitus was not demonstrated in SRS. Triglyceride levels, total cholesterol, LDL cholesterol and HDL cholesterol remained within normal ranges at baseline and 2 years after GH treatment. No cases of metabolic syndrome were diagnosed (80).

Table 1.7 Summary table on metabolic health and cardiovascular risk factors and GH treatment in SGA.

Study	**Metabolic health/cardiovascular risk factor and result**	**Duration of GH treatment**	**Subsequent change**
De Zegher et al (189)	Normal glucose concentrations.	2 years in all cases. Cessation of GH in 13/22 cases. Second GH treatment in 9/22: two years later (5/9) and three years later (4/9).	
Van Pareren et al (186)	Increased fasting glucose concentrations. Increased fasting insulin levels. Impaired glucose tolerance in 4%. HbA1c levels decreased. Systolic and diastolic blood pressures decreased. Total cholesterol, LDL cholesterol and HCL cholesterol each decreased.	6 years of GH treatment.	Six months after GH treatment cessation, glucose and insulin levels returned to baseline, impaired glucose tolerance was found in 10%, HbA1c levels were unchanged, systolic and diastolic BP unchanged. No change in total cholesterol. LDL and

Study	**Metabolic health/cardiovascular risk factor and result**	**Duration of GH treatment**	**Subsequent change**
			HDL cholesterol increased in girls only.
Hokken-Koelega et al (64)	Increased fasting insulin levels. Reduced systolic and diastolic blood pressure.	Increased fasting insulin at one and six years of treatment. Prevalence of impaired glucose tolerance decreased from 8% at baseline to 4% after six years of treatment.	Reduction in blood pressure maintained six months after treatment cessation.
Sas et al (185)	Reduced systolic and diastolic blood pressure.	Six years.	
Smeets et al (80)	Blood pressure unchanged at start and end of GH. Normal fasting blood glucose. Triglyceride, total cholesterol, LDL cholesterol and HDL cholesterol levels normal at baseline.	Fasting blood glucose increased on GH treatment	Two years after GH cessation, blood pressure stable, fasting blood glucose remained, and triglyceride and cholesterol levels remained normal.

1.13.3.3 Bone maturation

GH treatment is associated with advanced skeletal maturation (of previous delayed bone maturation) in a dose-dependent manner, however not to a level of advanced bone age (171, 190) and no difference has been found in children who receive a second course of GH compared to a single course (189).

1.13.3.4 Bone mineral density

Reduced birth size has been shown to have long-term negative effects on bone mineral content and bone mineral density (BMD) (68). Weight gain during infancy (70) and childhood (69) is a key factor in BMD accrual. Reduced bone mineral content has been demonstrated in SGA (64) and treatment with GH has been reported to increase bone mineral content (64, 191) and bone mineral density (192). When correction for bone size is performed, short SGA adolescents were shown to have comparable bone mineral apparent density (BMAD) to a reference population (192). The issue of true increase in BMD with GH treatment remains controversial; the increase may simply reflect a size effect.

1.13.4 Dosing, treatment schedules and monitoring

The dosing of GH depends on the patient diagnosis and size (either using weight or body surface area). The recommended dose in SGA is 35 mcg/kg/day (193). Measurement of IGF1 levels allows variation in response to be assessed.

GH is given at night to imitate physiological patterns and is principally prescribed daily (15). National guidance includes administration at a frequency of six or seven times per week (166, 193). The dose-dependent effects led to higher doses (66-100 mcg/kg/day) being prescribed in the past. Treatment is discontinued for the following reasons: height velocity <50% from baseline over the first year of treatment, near final height and height velocity <2 cm/year, final height achieved, or poor compliance which cannot be rectified (166, 193).

1.13.5 Short term side effects of growth hormone

Additional side effects of GH include headache, myalgia, arthralgia, reactions at injection sites, nausea and vomiting, visual problems, and paraesthesia (194). Rare but serious side effects of treatment include benign intracranial hypertension, which typically resolves after treatment withdrawal and tends not to recur on recommencement of treatment (195). Oedema and carpal tunnel syndrome have been reported in adults. In addition to direct side effects, slipped upper femoral epiphysis and exacerbation of pre-existing scoliosis may develop as a result of rapid growth (195). Slipped capital femoral epiphysis during growth hormone treatment has been reported in 2/62 SRS cases who had LOM H19/IGF2. These events were not considered by the treating physicians to have been related to GH and following surgical intervention, GH treatment continued (182). The same research group reported two cases of slipped capital femoral epiphysis in another paper (80). However, these are likely to have been the same two individuals

- both reports were of two females with the same molecular diagnoses and identical ages when affected (10 and 11 years old).

1.13.6 Potential long-term effects of growth hormone treatment and the SAGhE studies

Epidemiological studies suggested a relationship between raised serum IGF1 levels and its binding protein, IGFBP3 with risk of lung (196), breast (197), prostate (198), and colorectal (199) cancers. More recent studies have shown weak associations between raised IGF1 levels and higher risk of colorectal (200), breast (197) and prostate (201) cancers. As previously discussed, GH treatment is contra-indicated in children with conditions predisposing to malignancy such as Fanconi anaemia and Bloom syndrome (163).

A Health Technology Assessment systematic review in 2010 assessed evidence for GH treatment in children and found that few studies reported adverse events and none reported quality of life measures (178). There were no documented side effects observed in the treatment group of 37 people in a more recent study (46). The Safety and Appropriateness of Growth hormone treatments in Europe (SAGhE) study aims to evaluate long-term health in patients treated with recombinant growth hormone across the UK, France, Germany, Italy, Belgium, Switzerland, Sweden and The Netherlands. The French arm of the study commenced earlier and reported increased mortality in patients treated with GH compared to age- and sex-specific mortality rates in the French population. Significantly higher mortality rates were found in patients who had shorter stature at the start of treatment or who received GH doses >50mcg/kg/day. However, age at initiation of treatment, duration of treatment and peak GH levels were not associated with mortality risk. Mortality rates due to cerebral or subarachnoid haemorrhages and bone tumours were raised (6.7-fold and 5-fold respectively), although there was no overall increase in mortality secondary to all tumours (202). The authors acknowledged that higher mortality rates are found in adults with short stature therefore the use of the general French population as a comparison group may not be appropriate and analysis of specific causes of death is difficult given the low number of total events (n=93). Further results from the SAGhE study in Belgium, The Netherlands and Sweden were published in the same year and did not replicate the French findings. 76% of deaths were found to result from accidents or suicides, and none from malignancies or cardiovascular diseases. Furthermore, the deceased patients had not received GH doses in excess of 36 mcg/kg (203).

The French SAGhE study data was subsequently used to retrospectively evaluate cerebrovascular morbidity in patients who had been treated with recombinant growth hormone for isolated growth hormone deficiency or childhood short stature. The authors reported an association

between growth hormone treatment and increased risk of stroke in early adulthood (204). Mean GH doses were slightly lower than recommended for isolated GH deficiency. However, there were many limitations to the study and its interpretation: firstly, the sources used to identify events were incomplete although the authors attempted to correct for this and reported both observed events and estimated events. Secondly, there was a very low (45.5%) response rate to the morbidity questionnaire and bias of those responding cannot be excluded. Thirdly, no information was ascertained on confounding cardiovascular risk factors such as hypertension, diabetes, smoking or dyslipidaemia, which would grossly affect individual risk. Lastly, stroke rates were compared to stroke registries in Dijon, France and Oxford, UK, which are not comparable to a population with short stature or isolated GH deficiency.

A recent report of data from the SAGhE cohort, showed that there was no increased overall cancer risk in patients with isolated GH deficiency, idiopathic short stature and prenatal growth failure. GH treatment was not shown to affect the risk of cancer incidence or mortality in those whose initial diagnosis was not cancer (205).

Both the European Medicines Agency and the United States Food and Drug Administration questioned the validity of the results from the SAGhE study. The European Medicines Agency's Committee for Medicinal Products for Human Use highlighted that the general population had been used a reference group for the calculation of standard mortality ration which would lead to confounding and preclude the results being robust (206). The United States Food and Drug Administration 'identified a number of study design weaknesses that limit the interpretability of the study results' but did not explicitly state the weaknesses. Four medical publications were reviewed as well as reports from the Adverse Event Reporting System and these were assessed as not providing evidence to suggest a link between recombinant GH and an increased risk of death (207). Currently no change has been recommended in the prescribing of growth hormone.

In summary, GH improves height gain in SRS and there is some indication that this results in greater adult height. The beneficial effects on body composition in SGA with overall impaired glycaemic control do not appear to continue after treatment cessation. There is no evidence of long term adverse effect of GH on blood pressure or glycaemic control. There is no evidence that GH causes advanced bone age and effects on BMD remain controversial. There is little evidence specific to SRS meaning that decisions about treatment remain challenging. GH treatment is a safe treatment in the short term and long-term outcomes continue to be monitored.

1.14 Quality of life in short stature syndromes

1.14.1 Quality of life in short stature

When GH treatment first commenced, an aspect of the treatment rationale was improvement in quality of life. The World Health Organization (WHO) defines quality of life as 'individuals' perception of their position in life in the context of the culture and value system in which they live and in relation to their goals, expectations, standards and concerns' (208) and acknowledges this to be a wide-ranging and complex concept. Overall, quality of life is affected by an individual's physical and mental health, and social circumstances (208, 209) and can be modified by family situation, relationships, beliefs and socio-economic status (208, 209). Health-related quality of life (HRQoL) is multi-faceted construct that encompasses physical, emotional and social aspects of functioning and wellbeing (210).

A positive association between taller height and better mental health has been reported in adults (211, 212). Furthermore, a strong inverse association between height and suicide risk has been demonstrated in a Swedish cohort (213). Three cross-sectional studies have been performed in European countries assessing adult height in a sample of the population. HRQoL relating to mobility, self-care, usual activities, pain/discomfort, and anxiety/depression has been evaluated in Britain and height was demonstrated to be a significant predictor for quality of life. People with height SDS ≤-2.0 had the lowest quality of life scores with the main contributory factors being difficulties with pain/discomfort, mobility and usual activities (214). A French study enquiring about physical functioning, body pain, general perception of health, vitality, social functioning, mental health and role limitation relating to mental health found that height was a very weak predictor of HRQoL. A lower physical functioning score was reported with height below 149.2 cm in men and 136.0 cm in women (215). These heights would be compatible with adult height in SRS therefore this finding may be relevant to quality of life in SRS. An Italian study examined happiness scores and reported height was positively correlated with the highest happiness category (216).

In adolescents, a positive relationship has been demonstrated between height and wellbeing (217). Children and adolescents with short stature have lower health-related quality of life scores (209). The Wessex growth study showed that at age 12 years, shorter children had lower IQ scores, reading attainment levels, basic numerical skills, reduced internalisation of control and satisfaction with height. However shorter children in that study tended to have a lower social

class background and that accounted for much of the difference; in all factors except body satisfaction. Furthermore, even though children reported a desire to be taller, their self-esteem was not affected (218). In German adolescents, height did not influence HRQoL when confounding factors e.g. socio-demographic factors were corrected (219).

Children with marked short stature (height <140 cm or predicted adult height SDS <-2.5 were found to have significantly lower 'physical abilities' than other short children born SGA. HRQoL in short children born SGA was also shown to improve with GH treatment (220). The height reported of <140 cm would apply to SRS therefore it might be expected that similar findings would be present. The effects of GH in SRS might be similar to children born SGA, however this has not been demonstrated.

1.14.2 Quality of life in a short stature syndrome – Turner syndrome

To the author's knowledge there are no published studies of quality of life in SRS, however there is one study of the lived experience of SRS, which identified four themes: 1) appearance-related concerns; 2) strategies to deal with threats; 3) women's experiences of pain and disability; and 4) feeling disregarded in romantic relationships (221). Quality of life has been evaluated in Turner syndrome and will be discussed as an example of a disorder that causes short stature. Whilst there are additional features of Turner syndrome such as psychological and social difficulties, there are aspects related to height that might be informative. The aetiology of Turner syndrome is partial or complete loss of one X chromosome. The characteristic features are short stature and ovarian dysgenesis with a wide range of associated features including cardiac anomalies (particularly coarctation of the aorta), recurrent middle ear infections, specific learning difficulties (frequently mathematics), renal anomalies and absent or incomplete puberty (222). There are similar features in SRS. Girls with Turner syndrome are reported to be more socially vulnerable than their peers and display more internalising behaviour, social problems and immaturity (222), as well as reduced social activity/increased social isolation (222, 223).

HRQoL negatively correlates with both age at diagnosis and current age (223). HRQoL has also been shown to be better in women who were content with their height (224). Girls with Turner syndrome have lower scores in the 'physical functioning' dimension of HRQoL (225) although increased height has been positively correlated with physical functioning (224, 225). In contrast, another study failed to demonstrate a correlation between overall HRQoL and height (226). Body mass index is not associated with HRQoL (223, 226).

HRQoL is not related to the number of stigmata of Turner syndrome (223) however, women who are anxious about their physical appearance have been shown to have lower HRQoL in the 'social functioning' domain (224) and a negative association has been described between 'vitality' and the presence of skeletal anomalies (225). Bone mineral density has been both positively correlated with HRQoL (223) and negatively correlated with 'vitality' and 'physical functioning' (225).

There is a negative association between the social functioning aspect of HRQoL in those with post-menarchal status compared to girls in puberty without menarche (225) however age at onset of puberty does not significantly affect HRQoL (224). Reduction in quality of life related to infertility approaches double that attributed to short stature (227).

Women who were treated with GH report better HRQoL scores in 'daily activities', 'aggressive emotions', 'social functioning', 'role limitations due to emotional problems', and 'bodily pain' compared to the normal population (224). Reduced pain has been associated with GH treatment in Turner syndrome (223). Two studies have found no correlation between HRQoL and GH treatment in Turner syndrome (225, 226), although one demonstrated a negative correlation between age at initiation of GH treatment and the 'vitality' aspect of HRQoL but no relationship with GH treatment duration (225).

In summary, height is positively associated with increased quality of life in adults although in the children, there is less evidence that the same applies. Turner's syndrome was discussed as an example of a condition causing short stature where there is evidence on HRQoL. Women with Turner's syndrome are reported to have reduced physical functioning. There are similarities in the phenotypes of Turner syndrome and SRS – such as short stature, skeletal manifestations, cardiac abnormalities, renal anomalies and specific learning difficulties. However, there are clear differences – such as absent/delayed puberty, infertility – which makes fully applying the Turner syndrome research to SRS inappropriate. Furthermore, Turner syndrome affects females exclusively whereas SRS affects both genders. There may be different quality of life outcomes in males and females with SRS. In addition, quality of life outside health-related aspects might be affected and has not been evaluated.

1.15 Conclusions

This review of the literature has refined my understanding of SRS as a clinical condition with the key feature of pre- and postnatal growth restriction. Growth patterns, dysmorphic features, feeding difficulties have been reviewed and where possible evidence on body composition,

metabolic effects and the lifecourse have also been reviewed. The genetic mechanisms have been elucidated in up to 60-70% of SRS patients, however the aetiology of SRS remains idiopathic in some cases. GH treatment and quality of life have been reviewed and unanswered questions discussed with the paucity of data specific to SRS highlighted.

The review has also identified gaps in the understanding of the long-term outcome of individuals with SRS as studies have focussed on childhood and particularly height. There is, therefore, a need to understand the long-term outcomes in adults of the clinical phenotype and whether this differs from childhood as well as body composition, height, quality of life and whether prior GH treatment influences these outcomes.

Chapter 2 Aims and hypotheses

The study aims to:

1. Describe the adult phenotype of SRS, including descriptions of height, body composition, metabolic health and health outcomes.
2. Compare the adult phenotype to the well-established childhood phenotype and clinical diagnostic scoring systems.

Compare adults with molecularly confirmed SRS who were not treated with GH to those who were treated with GH in order to:

3. Establish whether or not prior GH treatment affects final height, body composition and metabolic health.
4. Describe quality of life in these cases and evaluate whether or not GH treatment is associated with improved quality of life.

The hypotheses of this study are:

1. The adult phenotype of SRS differs from the childhood phenotype.
2. Growth hormone treatment in childhood increases final height and improves body composition in SRS.
3. Previous growth hormone treatment is associated with improved quality of life in adults with SRS.

Chapter 3 Methods

3.1 Overview of methods

This thesis identifies and studies a cohort of individuals with molecularly confirmed SRS aged ≥13 years and analyses data across multiple parameters to address the aims and hypotheses of the study. I arranged for each participant to attend a study appointment in order to conduct a medical history, full clinical examination, an assessment of body composition, blood tests (± skin biopsy) and completion of quality of life questionnaires. This research was conducted within a wider study entitled the 'Study of Adults and Adolescents with Russell-Silver syndrome' (STAARS), which also included a qualitative study of a subset of the participants. Full details of these methods will follow.

3.2 Study preparation

3.2.1 Funding

Funding for the 'Study of Adults and Adolescents with Russell-Silver syndrome' (STAARS) was obtained from the National Institute for Health Research (NIHR) (grant number PB-PG-1111-26003) under the category of Research for Patient Benefit. This funding was secured by Professor Karen Temple (University of Southampton and University Hospital Southampton, Southampton, UK), Dr Justin Davies (University of Southampton and University Hospital Southampton, Southampton, UK), Professor Hazel Inskip (University of Southampton and University Hospital Southampton, Southampton, UK), Professor Christopher Byrne (University of Southampton and University Hospital Southampton, Southampton, UK), Dr Angela Fenwick (University of Southampton, Southampton, UK), and Mrs Jenny Child (Child Growth Foundation membership secretary, Birmingham, UK) prior to my commencing work on the study. The study steering committee was comprised of the aforementioned along with Dr Emma Wakeling (North West Thames Regional Genetics Service, London, UK) and Dr Renuka Dias (Birmingham Women's and Children's Hospital, University of Birmingham, Birmingham, UK).

3.2.2 Development of Study Documentation

The study protocol and supporting documents are listed in Table 3.1.

Table 3.1 List of documents, as submitted for ethics review.

Document	*Version*	*Date*
Covering Letter		19 November 2013
Interview Schedules/Topic Guides	Telephone Prompt Form, v1	08 November 2013
Interview Schedules/Topic Guides	Doctor-managed record of history and exam with RSS proband, v1	08 November 2013
Interview Schedules/Topic Guides	In-Depth Interview Framework, v1	08 November 2013
Investigator CV	Prof Temple	19 November 2013
Letter from Sponsor		20 September 2013
Letter of invitation to participant	Response Form, v1	08 November 2013
Other: Letter from Funder - NIHR Correspondence		16 July 2013
Other: Participant Detail Form	1	08 November 2013
Other: Study Visit Outcome Sheet	1	08 November 2013
Participant Consent Form: Participating Adult	1	12 November 2013
Participant Consent Form: Parent Consent Form	1	12 November 2013
Participant Consent Form: Assent Form	1	08 November 2013
Participant Consent Form: Participating Relative	1	08 November 2013
Participant Consent Form: In-depth Interview Participating Adult	1	08 November 2013
Participant Consent Form: In-depth Interview Assent Form	1	08 November 2013
Participant Consent Form: In-Depth Interview Parent Consent Form	1	08 November 2013
Participant Information Sheet: Adults/Parents and Guardians Information Booklet	1	08 November 2013
Participant Information Sheet: Young People	1	18 November 2013
Participant Information Sheet: Participating Relative	1	08 November 2013
Participant Information Sheet: In-depth Interview Information Booklet - Participants and Parents	1	08 November 2013
Participant Information Sheet: In-depth Interview Information Sheet for Young People	1	08 November 2013
Protocol	1.1	08 November 2013
Questionnaire: Sheehan Disability Scale		
Questionnaire: Schedule for the individual evaluation of QOL-direct weighing		
Questionnaire: Male puberty self-assessment questionnaire	1	08 November 2013
Questionnaire: Female puberty self-assessment questionnaire	1	08 November 2013
Questionnaire: Early Medical History Questionnaire for parent of STAARS participant	1	08 November 2013
REC application	132544	11 December 2013
Referees or other scientific critique report	NIHR Lay and Peer Review Forms	

Development of the study documentation commenced prior to the work undertaken for this research thesis. I was significantly involved in developing and editing all the final documents submitted for ethics review. The puberty self-assessment questionnaires for females and males had been validated for previous research and were reproduced without alteration after approval by the study steering committee. The wellbeing questions were designed by the steering committee and all quality of life assessments were piloted by Professor Temple. The Sheehan Disability Scale is a validated tool for the assessment of disability. The Schedule for the Evaluation of Individual Quality of Life – Direct Weighting (SEIQoL-DW) is a standardised assessment tool. Both the Sheehan Disability Scale and the SEIQoL-DW are described in section 3.8.1. The in-depth interview framework was designed by the study steering committee.

The following documents were designed by the study steering committee and I adapted them during the work for this thesis:

i) the 'doctor-managed record of history and examination' with each SRS proband;

ii) the 'telephone prompt' form was updated when details of the study appointment had been finalised, including the requirements for 12 hours of fasting and abstention from exercise and alcohol for the 24 hours prior to bio-electrical impedance analysis.

iii) the 'participant details' form was first designed by the study steering committee and was modified by me in order to include details of each study identification number. The method for generation of study identification numbers was proposed by me and discussed with the steering committee.

iv) the three information booklets: 1. for adult participants or parents of participating children; 2. for younger participants; and 3. for relatives of participants; these were first developed by the steering committee and subsequently modified.

v) the questionnaire on early medical history for parent of STAARS participant was also modified.

The following documents were designed independently by me and reviewed by the study steering committee:

i) the response form

ii) the study visit outcome sheet

iii) the consent forms

iv) the assent forms.

3.2.3 Acceptability of study protocol and questionnaires to people with SRS

I organised for the study protocol, information sheets and consent forms to be reviewed by a patient support group (the 'Teens and Adults with Russell-Silver syndrome' (TARSS) group, which is affiliated to the Child Growth Foundation (CGF). Improvements were suggested and the group confirmed that they did not feel the study requirements would place excessive burdens on the participants or their families.

3.2.4 Research and development approvals

I organised the following, and with Professor Temple, led discussions with staff from the Research and Development office, University Hospital Southampton (UHS). I applied for study sponsorship through UHS NHS Foundation Trust. Provisional and then full research sponsorships were granted confirming that UHS agreed responsibility for the quality and conduct of the research study and that appropriate indemnity arrangements were in place.

Approval from NHS Research and Development was sought via the 'National Institute for Health Research Coordinated System for gaining NHS Permission' (NIHR CSP). The standard process for Research and Development approval involves application at each individual hospital site where research is to be performed. In rare disease research, the bureaucracy involved is frequently excessive in comparison to the number of individuals affected by the disease and who might be recruited to studies. In acknowledgement of this and with a view to improving research processes, the NIHR UK Rare Genetic Disease Research Consortium Agreement (Musketeer Memorandum) was established in 2013. This agreement amongst the 23 genetics centres in the UK means that full Research and Development local and global checks are performed at the lead site whereas at other research sites or patient identification sites, the checks are not repeated to the same degree. Approval at centres other than the lead site should be granted within 3 days of lead site approval. In order to implement this process, I liaised with the Lead Genetics Research coordinator for Rare Disease, Dr Gillian Borthwick (National Research Coordinator, NIHR Genetics Specialty Group, Institute of Genetic Medicine, Newcastle) regarding study design. To avoid cost implications for the other research sites and gain approval under the Musketeer, the following modifications to the study protocol were required: at centres outside UHS the only samples to be collected were blood samples; participants could only be seen at the Genetics centre and not in clinical research facilities.

I attended a training course on the use of the online 'Integrated Research Application System' through which applications to both NIHR CSP and the National Research Ethics Service (NRES) are processed. I entered the relevant details about the study design into the web-based electronic system which ensures that essential information is complete and allows multiple forms to be populated from a single-entry source. I then liaised with Dr Gillian Borthwick (Newcastle), in order to finally approve the application and complete the required information regarding the other Genetics centres. It was necessary for the team in Newcastle to communicate with the UHS Research and Development department, however this caused significant delay (owing to lack of prioritisation of this project over others) and I regularly liaised with both parties in order to expedite the process. Research and development approval was granted at UHS in May 2014 (RHM NEU 0209).

To facilitate participation in the study, research visits were to be offered in sites local to participants. In order to undertake work at other NHS Trusts, as an existing NHS employee, a 'letter of access' is required for each site to be visited. The following were required to apply for such access at each individual Trust:

- confirmation from the employing Trust that pre-employment checks had been performed
- copy of employment contract

- confirmation of training in 'Good Clinical Practice'
- research curriculum vitae in the template provided by NRES.

My initial plan was to apply for letters of access only where necessitated by participants being identified and wishing to be seen at centres outside UHS. I later modified this plan to apply at all centres in order to be able to attend in advance of conducting study visits. This, therefore, permitted searching clinical databases at other genetics centres and sending study information to their patients.

3.2.5 Ethics approvals

I completed the online application form and reviewed my proposed responses with Professor Karen Temple and Dr Justin Davies. I contacted other required signatories – the head of UHS Research and Development and an expert in ionising radiation (in order to explain and approve the use of the imaging tests). I submitted the online application and arranged a date to attend the NRES committee meeting. The ethics application was reviewed by NRES Committee South Central – Hampshire B on 11 December 2013. I attended the meeting along with Professor Karen Temple and Dr Justin Davies and explained the application. Minor amendments were recommended and after these contentious points were either altered or justified, full ethical approval was granted (REC reference: 13/SC/0630) in January 2014.

Additional registration of the research project was required by the University of Southampton. I applied for this via the Ethics and Research Governance Online (ERGO) system. All study documents were submitted. Full University ethics review was not required because NHS ethical approval had been granted.

3.3 Recruitment methods for study participants

In view of the rarity of SRS and the absence of a registry, multiple methods of recruitment were employed in order to contact potential participants:

1. Members of the CGF and the affiliated TARSS group were informed about STAARS at annual conferences – by Professor Temple in 2012 and by myself and Professor Temple in 2013, 2014 and 2015. I liaised with the membership secretary of the Child Growth Foundation (Mrs Jenny Child) to further disseminate information about STAARS. I organised for information about STAARS to be included in Child Growth Foundation newsletters and via social media by the TARSS organiser. Information booklets and response forms were posted to adult members or parents whose children were an appropriate age by the CGF membership secretary Mrs Jenny Child. In order to maintain confidentiality these 36 members/families were contacted directly by Mrs Child.

Several potential participants identified via this route had not previously undergone molecular diagnostic testing. If individuals expressed an interest in participating in the study, I contacted them by phone. I explained the diagnostic testing and arranged testing by preparing and posting to interested participants a pack containing a completed form, the necessary collection bottle, and an addressed, padded envelope. Potential participants then arranged venepuncture at their GP surgery and the sample was sent to the Wessex Genetics Regional Laboratory for analysis. I then followed-up the results and re-contacted these individuals subsequently.

2. An ongoing national study led by Professor Temple, called 'Imprinting Disorders: finding out why' includes participants with SRS. Those who had expressed an interest in further research were contacted in writing and sent an information booklet. With the study research assistant, I searched the 'Imprinting Disorders: finding out why' database for individuals with SRS who were aged 13 and above. I then reviewed the consent form of each participant identified to confirm he/she had agreed to be contacted about further research. I drafted a letter explaining STAARS to send out and reviewed this with Professor Temple before confirming the final version.

3. Patients treated at Wessex Clinical Genetics Service were identified by review of the clinical database. I searched the clinical database for 'Russell-Silver syndrome' and 'Silver-Russell syndrome' and limited the results by the age of the patient to find those born after 2001. The search term included possible SRS and confirmed. I then examined each patient's file to review the details of their diagnosis. Where physical files were no longer stored, I reviewed electronic scanned files. I subsequently contacted the patients' consultants who either telephoned or sent letters and information booklets directly to their patients.

4. I attended the Wessex Regional Genetics Laboratory and after being provided with a list of patients of the correct age with positive results identified from the database, I retrieved and reviewed each result. I searched for the correct address for each clinician, and after I drafted letters and finalised these with Professor Temple, I posted them to the relevant consultants (at centres outside Wessex) requesting that the appended study information be passed on to their patient(s).

5. I contacted each UK Genetics centre regarding STAARS – initially by e-mail and subsequently by both e-mail and telephone. Methods for recruitment at centres outside Wessex differed; some were able to review clinical databases for patients with a diagnosis of 'Russell-Silver' or 'Silver-Russell' syndrome, others searched within letters or relied on clinician recall. Both myself and Professor Temple have given oral communications about STAARS at national conferences to promote the study to Clinical Genetics teams. I was invited to deliver a presentation at the British

Society for Genetic Medicine annual meeting in September 2014. This enabled me to explain and promote the research study.

6. At sites where Research and Development approval was granted via the Musketeer Memorandum, consultants in Paediatric Endocrinology were contacted by Dr Davies. Where patients with SRS aged 13 years and above were identified, I sent packs including information booklets and response forms in order for the local team to post directly to their patients.

Following either contact from interested individuals or the return of response forms to me, I contacted each participant by telephone to explain the study in detail, ensure they had received the study information booklet and answer any questions. The telephone prompt form was implemented. I posted information booklets if not already received. Where the diagnostic testing confirmed a molecular diagnosis of SRS, a study appointment was offered at a date and location suitable for the participant. Aside from three early participants, in cases where molecular testing was negative, this was explained and involvement in this study was precluded.

In order to arrange appointments at UHS, I liaised with the participant, the Genetics Research nurses, the Clinical Research Facility (for room bookings), the Clinical Research Facility laboratory (to ensure that samples were expected and processed appropriately), the Osteoporosis Centre (to arrange imaging tests), and the Respiratory technicians (to perform spirometry). To arrange appointments at other Genetics centres, I liaised with the participant, the local Genetics team – to arrange a clinic room and laboratory support with samples. I produced standard operating procedures for blood and tissue sampling, which covered:

- the correct type and number of blood bottles
- the order of draw for blood bottles used during venepuncture
- identification (forename, surname, date of birth and address or NHS number)
- the correct labelling for research laboratory samples – STAARS identification number only
- the schedule for samples to be sent directly to NHS pathology, samples to be centrifuged and the appropriate parameters, samples to be frozen at -70 ^{o}C within two hours of sampling and samples to be transported (by me) from centres outside UHS

3.4 Clinical assessment

I recorded history and examination findings within the 'Doctor-managed record of history and examination with SRS proband' (see Appendix F). The clinical assessment of each participant was comprehensive and included thorough routine clinical examination of respiratory, cardiovascular, gastro-intestinal and neurological systems. Assessment of dysmorphology was conducted and

standard terminology applied for the head and face (228), hands and feet (229), lips, mouth and oral region (230), nose and philtrum (231), and ear (232).

3.5 Methods for measurement of blood pressure

In view of the variable nature of blood pressure (BP) (233), measurement was standardised to improve reproducibility. Blood pressure measurements were taken with an automated machine where available (by myself or a research nurse), and manually (by me) if this was not possible. A stronger correlation has been shown between automated blood pressure readings and awake ambulatory blood pressure measurement (gold standard) than manual blood pressure measurement and awake ambulatory blood pressure measurement (234). Lower systolic and diastolic blood pressure measurements have been reported with the use of automated compared to manual blood pressure measurement (235). Therefore, the approach of using automated blood pressure measurement where possible would improve the accuracy of the results obtained in the studies presented in this thesis.

The first reading was measured after three minutes of rest with the participant lying on an examination couch with the head of the bed at a 45^{o} angle. Participants were asked not to engage in discussion during this time. Two further readings were taken for each patient and the mean of the second and third readings used for data analysis. Where blood pressure readings remained elevated, this was explained to the participant. The study appointment was continued and repeated measurements were attempted at the end of the consultation. If these remained elevated, further repetition of the process was not undertaken. In those cases, participants were advised to make an appointment with their GP practice nurse to have a blood pressure reading taken at another date.

3.6 Evaluation of metabolic status

3.6.1 Blood tests

Following 12 hours of fasting, venepuncture was performed at the start of each study appointment (by a research nurse or by me). Blood samples were sent to NHS diagnostic pathology laboratories for concentrations/counts of the following: haemoglobin, white blood cells, platelets, sodium, potassium, urea, creatinine, total protein, albumin, alanine aminotransferase, bilirubin, alkaline phosphatase fasting blood glucose, triglycerides, cholesterol – including total, low-density lipoprotein and high-density lipoprotein, glycosylated haemoglobin

(HbA1c), thyroxine (T4) and thyroid stimulating hormone. During the study period, UHS pathology laboratory began reporting non-LDL cholesterol in addition to the aforementioned.

A full blood count was included in order to gauge participants' general health. Detection of anaemia or raised white blood cell counts could assist in the interpretation of other blood tests results or clinical examination findings.

The initial study plan was to postpone testing of insulin-like growth factor 1, insulin and C-peptide so that rather than being tested after each study appointment, batch testing would be performed a later date. From March 2015, after discussion at a study steering committee this process was altered so that all testing was requested at the time of venepuncture.

Table 3.2 shows the methods used for analysis of blood tests in the NHS diagnostic laboratory at University Hospital Southampton. Beckman Coulter (Beckman Coulter (UK) Ltd, High Wycombe, UK), Glenbio (Glenbio Ltd, Co Antrim, UK), Sebia (Lisses, France) and Siemens (Siemens Healthcare Limited, Camberley, Surrey, UK) equipment and assays were used.

Table 3.2 Tests performed in the University Hospital Southampton NHS diagnostic pathology laboratory. BCP = bromcresol purple dye.

Test	**Company**	**Analyser**	**Analysis method**
Alanine aminotransferase	Beckman Coulter	AU5800/AU680	Kinetic rate
Alkaline phosphatase	Beckman Coulter	AU5800/AU680	Kinetic rate
Albumin	Glenbio	AU5800/AU680	Dye-binding (BCP)
Total bilirubin	Beckman Coulter	AU5800/AU680	Photometric colour
Total protein	Beckman Coulter	AU5800/AU680	Photometric colour
Cholesterol	Beckman Coulter	AU5800/AU680	Enzymatic colour
Triglycerides	Beckman Coulter	AU5800/AU680	Enzymatic colour
Glucose	Beckman Coulter	AU5800/AU680	Enzymatic

Test	Company	Analyser	Analysis method
HbA1c	Sebia	Sebia capillarys	Capillary electrophoresis
Thyroid stimulating hormone	Beckman Coulter	UniCel DxI	Chemiluminescent immunoassay
Free T4	Beckman Coulter	UniCel DxI	Chemiluminescent immunoassay
Insulin	Beckman Coulter	UniCel DxI	Access Ultrasensitive one-step immunoenzymatic
C-peptide	Siemens	Immulite 2000	Chemiluminescent immunoassay
IGF1	Siemens	Immulite 2000	Chemiluminescent immunoassay

I met with the Pathology lead for Clinical Trials in order to establish a request form for STAARS under a specific research code. This form also enabled testing of STAARS participants seen outside UHS using NHS numbers rather than hospital numbers.

With participants' consent and where possible, serum and plasma from each participant, was centrifuged and frozen at -70 ^{o}C within 2 hours for later testing. At a later date, samples were thawed and tested for aspartate aminotransferase and gamma glutamyl transferase.

3.6.2 Derived measures of metabolic status

Waist-to-hip ratios were calculated as follows: waist circumference [cm]/hip circumference [cm]. Body mass index was calculated as: weight [kg]/height [m]^2. Weight status in individuals aged ≥18 years was categorised by BMI using the World Health Organisation classification (236):

Underweight = BMI <18.5 kg/m^2

Ideal weight = BMI 18.5 to 24.99 kg/m^2

Overweight = BMI 25 to 29.99 kg/m^2

Obese = BMI ≥30 kg/m^2

Obese class I = BMI 30 to 34.99 kg/m^2

Obese class II = BMI 35 to 39.99 kg/m^2

Obese class III = BMI ≥40 kg/m^2

Metabolic syndrome was evaluated using the harmonised definition agreed by the International Diabetes Federation Task Force on epidemiology and prevention; the National Heart, Lung and Blood Institute, The American Heart Association, the World Heart Federation, the International Atherosclerosis Society and the International Association for the Study of Obesity (237). According to this definition, elevated BP was defined as a mean systolic ≥130 mmHg and or mean diastolic ≥85 mmHg. Elevated waist circumference according to population- and country-specific definitions; ≥94 cm and ≥80 cm in Caucasian men and women respectively, and ≥90 cm and ≥80 cm in Asian men and women respectively. Elevated triglycerides were defined as ≥ 1.7 mmol/L or pharmacological treatment for elevated triglycerides. Reduced HDL cholesterol was defined as <1.0 mmol/L in males and <1.3 mmol/L in females or pharmacological treatment for reduced HDL cholesterol. Elevated fasting blood glucose was defined as ≥100 mg/dl. Where any three of the above five risk factors were present metabolic syndrome was diagnosed.

In children, blood pressure is positively correlated to height therefore standard blood pressure tables adjust for sex, age, and height centile (238). In adults, the negative association between stature and systolic blood pressure (i.e. greater height associated with lower blood pressure) has been postulated as a mechanism linking short stature and cardiovascular disease risk (239).

The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as follows: fasting insulin [mU/L] x fasting glucose [mmol/L]/22.5 (240). The quantitative insulin sensitivity check index (QUICKI) was calculated as follows: 1/(log fasting insulin [mU/ml]+log fasting glucose [mg/dl]) (241).

IGF1 SDS were calculated using the formula:

$$SDS = ([y/M]^L - 1) / (S \times L)$$

where y = the IGF1 measurement and M, L and S equal the age- and sex-specific values in the reference data. Reference values were taken from Bidlingmaier et al (2014) which includes normative data on 15 014 individuals from Europe, Canada and the USA (242).

3.7 Evaluation of body composition

In order to improve reliability and accuracy of anthropometric measurements, the process for each measurement was standardised and the same methods were used to identify landmarks (described below).

3.7.1 Measurement of height

Height measurements were undertaken by a research nurse and myself using a two-person technique according to a standard operating procedure: the participant stands facing the examiner, the participant's heels were placed against the stadiometer or wall (unless they were unstable in this position). The head was positioned in the Frankfurt plane – that is when the lower border of the lower canthus of the eye is in line with the external auditory meatus (Figure 3.1).

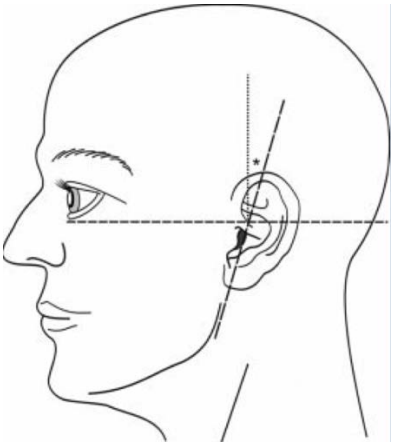


Figure 3.1 The Frankfurt plane. The horizontal dashed line shows the position of the Frankfurt plane (232).

The Frankfurt plane has been acknowledged as a pragmatic compromise for studying skulls, however its position in living subjects is normally distributed a true extracranial horizontal axis and there are limitations to its ability to determine the natural head position (243). Biological variation in craniofacial features contributes to the differences in position of the Frankfurt plane therefore dysmorphic craniofacial features might increase this variation. However, a PubMed search on the 'Frankfurt plane' or 'Frankfort plane' and 'dysmorphic' did not reveal publications in this area.

Upward pressure was applied to the mastoid process and each participant asked to inhale and relax the shoulders. An assistant then read the measurement on the stadiometer. For participants measured at University Hospital Southampton, the Marsden HM-250 Leicester height measure was used (Marsden, Henley-on-Thames, Oxfordshire, UK). The height measurement was

repeated after the participant had stepped away from the stadiometer and been prepared again. Measurements were deemed acceptable if the two readings were within 2 mm of each other. If this was not the case, further measurements were repeated until two were within 2 mm of each other. This procedure followed the standard operating procedure at the NIHR Southampton Biomedical Research Centre (244). At other research sites, the equipment available for measurement of height was used. These conformed to NHS standards for equipment.

3.7.2 Derived measures of height gain

Height calculations were performed as follows:

Height gain on GH = height SDS at end of treatment – height SDS at start of treatment.

Early height SDS was obtained from growth measurement at the start of GH treatment or between age 2-5 years.

Total height gain = final height SDS – early height SDS.

Target height was calculated using the formula: (maternal height in cm + paternal height in cm)/2 with 6.5 cm subtracted for female participants and 6.5 cm added for male participants (32).

Distance to target height = target height SDS – final height SDS

3.7.3 Measurement of weight

For participants measured at University Hospital Southampton, weight was measured using Seca weighing scales (Seca, Hamburg, Germany). Initially weight measurement was repeated three times. However, after it was noted that the readings were identical to two decimal places, the process was reviewed and amended to a single measurement. These are Class III scales, which have a maximum error allowance of 0.1 kg. Outside UHS, it was necessary to use the available weighing scales, however all departments conformed to NHS standards for equipment.

3.7.4 Measurements of skin fold thickness and limb circumferences

Measurements of skin fold thickness have been performed in individuals with SRS (14, 25, 184) and specifically for the estimation of total body fat in SRS (245).

I performed measurement of skin fold thickness using Holtain calipers (Holtain Ltd, Crosswell, Crymych, Pembrokeshire, UK) using a standardised approach (246). Measurements were taken at four sites on the non-dominant side of each participant irrespective of body asymmetry: biceps, triceps, subscapular, and supra-iliac skin folds. A mark placed half way between the acromion and the olecranon was used as the level for which to gather the biceps skin fold anteriorly and the

triceps skin fold posteriorly. The subscapular skin fold was identified by identifying the most inferior and medial aspect of the scapula and marking 1 cm medial and 1 cm inferior to that landmark. The landmark for the supra-iliac skin fold was 1 cm superior and 2 cm medial to the anterior superior iliac spine. All skin folds were gathered by grasping the skin from a distance of 7-8 cm then bringing the thumb to the index and second fingers to hold the skin and subcutaneous fat separate from the underlying muscle. All measurements were repeated twice in rotation and a third measurement taken if the second measurement was not within 7.5% of the first. All measurements were taken to an accuracy of 0.1 cm using a metal tape measure, in order to prevent stretching which is possible with a cloth tape measure. It was ensured that the measuring tape was resting on but not indenting the skin.

- For the mid-upper arm circumference, the acromion and the olecranon were marked with the elbow flexed at 90^{o}. The measurement was then taken with the arm in full extension at the point half way between the two marks.
- For thigh circumference, the anterior superior iliac spine and the proximal aspect of the patella were marked and the measurement was taken at a point half way between the two.
- Waist measurement was performed by marking the lower border of the ribs in the mid-axillary line bilaterally as well as the superior aspect of the iliac crests bilaterally. At each side, the distance between the two marks was measured and a cross marked at the points equidistant from the two marks. Waist measurement was taken by measuring the circumference around the torso with the tape measure passing through both crosses. Participants were asked not to deliberately hold their breathing in or out. After asking the participant to inhale and then exhale, waist measurement was taken during the pause after exhalation.
- The correct placement for hip measurement was assessed by visually assessing the location of widest part of the hips and the lower buttock. This point is said to lie between the greater trochanters.

3.7.5 Derived measures of body composition

Skinfold thicknesses were used to estimate body fat. For participants aged <18 years, triceps and subscapular skinfolds were used to estimate body fat percentage according to the Slaughter calculations (247). For participants aged ≥18 years, the sum of the mean measurements for biceps, triceps, subscapular and supra-iliac skinfolds was calculated and equivalent fat content (percentage of body weight) obtained from the appropriate sex and age group in the reference data of Durnin and Womersley (248).

Limb circumference measurements were used to assess for body asymmetry. Waist circumference measurements were used to calculate waist-to-hip ratios and in the evaluation of metabolic syndrome (see section 3.6.2). Cut-off values for waist-to-hip ratios were obtained from a UK study of 1918 men and women (249). The harmonised definition of metabolic syndrome was used (as agreed by the International Diabetes Federation Task Force on epidemiology and prevention; the National Heart, Lung and Blood Institute, The American Heart Association, the World Heart Federation, the International Atherosclerosis Society and the International Association for the Study of Obesity) (237).

3.7.6 Bio-electrical impedance analysis

Bio-electrical impedance analysis (BIA) is a technique used to calculate total body water by measuring the impedance to flow of current, caused by the body. The underlying principle is that conduction of electricity is better through the lean compartment of the body – comprising muscle, bone and water – than the fat compartment, which is low in body water. This gives information on:

- extra-cellular, intra-cellular and total body water
- body fat and fat weight
- body lean mass and dry lean mass

I performed BIA using the Bodystat Quadscan 4000 (Bodystat Ltd, Ballakaap, Ballafletcher Road, Cronkbourne, Douglas, Isle of Man, IM4 4QJ, British Isles) using a standardised approach (250). I calibrated the machine using the supplied calibrator at the start of each day of use. All calibration measurements were within acceptable limits. Pregnancy was an exclusion criterion to BIA.

BIA is affected by hydration status including alcohol and caffeine intake (251). Each participant was therefore contacted during the week prior to their study appointment and requested that he/she abstain from alcohol for 24 hours before the appointment and from caffeine on the day of the appointment. Furthermore, participants were asked to abstain from strenuous exercise for the 24 hours before the appointment. BIA was conducted after anthropometric measurements had been completed. Participants were asked to remain supine for 5 minutes prior to BIA measurement.

Two electrodes were placed on the right foot and right hand. The position of the foot electrodes is one behind the second toe and the other on the ankle between the medial and lateral malleoli. One hand electrode was placed behind the metacarpo-phalangeal joints and the other across the radial and ulnar styloid processes (see Figure 3.2).

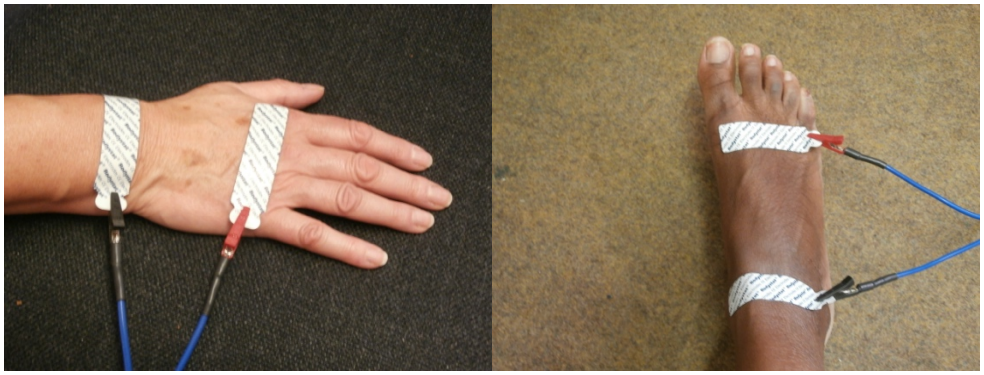


Figure 3.2 Placement of hand and foot electrodes for bio-impedance analysis.

All electrodes were positioned with the connector on the side closest to the researcher. Each of the two leads for connection to the Bodystat Quadscan machine combines two leads which have one red and one black alligator clip attached. One lead was used for the hand and the other for the foot: the red alligator clips were attached to the distal electrodes; the black to the proximal ones.

Limbs were positioned so that they were not in contact with any other part of the body and this was confirmed before analysis commenced. Participants were requested to refrain from speaking, coughing or sneezing (as much as possible) or any other voluntary muscle contraction during the measurement period. In addition to test results, the machine number and test number were recorded.

3.7.7 Dual energy x-ray absorptiometry

Dual energy x-ray absorptiometry (DXA) is used to measure bone mineral accrual, bone loss and body composition. The technique uses two x-ray beams of different energy levels which are attenuated differentially by different types of tissue present in the subject; high-energy beams are attenuated by bone and low-energy beams are attenuated by soft tissue (fat and muscle). DXA has primarily been used to measure bone mineral density (BMD) in order to evaluate osteoporosis and estimate fracture risk. DXA is a highly accurate and precise method of quantifying BMD and mass in vivo. A DXA image is a two-dimensional projection that combines the low and high energy attenuations and because the calculated measurements are taken from two-dimensional images projecting a three-dimensional structure, the depth of the bone cannot be evaluated. Areal bone mineral density (aBMD) is calculated from bone area and reported in units of g/cm^2

(252). aBMD measurements are size dependent and result in under-estimation in small individuals, which is relevant to individuals with SRS who have short stature. However, volumetric density (known as bone mineral apparent density, BMAD) can be calculated to correct for the effects of bone size. Although this correction is not complete, BMAD provides a better reflection of volumetric density (253, 254) and is usually calculated for specific bone sites such as the lumbar spine.

There are some limitations to DXA analysis: firstly, only two tissues can be assessed simultaneously. This means that soft tissue analysis can only be calculated in areas excluding bone. Secondly, there is a lack of standardisation and accuracy between manufacturers.

Age- and sex-specific standard deviation scores (known as Z-scores) can be calculated for aBMD by comparison to a reference database. Z-scores are used in children to diagnose low bone mass. T-scores are calculated by comparing an individual's aBMD to a young reference (at peak bone mass) and expressing the difference in units of the population SD. T-scores are useful in adults rather than children and are inversely correlated to fracture risk (252).

DXA scans were performed by staff at the Osteoporosis Centre at UHS using a Hologic Horizon W instrument (Hologic Inc., Bedford, Massachusetts, USA) with APEX v 5.5.3.1 software. Equipment is calibrated with quality control checks before every use using a spine phantom. A weekly step-wedge check is performed for system accuracy. The manufacturer's coefficient of variation for the instrument was 0.75% for whole body scans in adults. Scans were performed with participants in light clothing and with metal objects removed (e.g. metal zips or buttons). Pregnancy was an exclusion criterion.

The following scans were performed: whole body, lumbar spine, and hip. The standard procedure is to scan the hip on the non-dominant side. The presence of asymmetry, reflects mosaicism, which would be compatible with abnormal growth on the smaller side. I proposed to the STAARS steering committee that scans should be performed on the smaller side in cases of asymmetry – in order to target tissue that is more affected. The committee agreed and DXA was performed on the non-dominant side or the smaller side where asymmetry was present. Scans were analysed using the Whole Body Fan Beam Analysis algorithm. The measurements collected were:

- fat mass, fat mass index, fat-free mass, fat-free mass index, lean mass, lean mass index, percentage fat of the left arm, right arm, trunk, left leg, right leg, subtotal (excluding the head), head and whole body
- areal BMD at the left arm, right arm, left ribs, right ribs, thoracic spine, lumbar spine, pelvis, left leg, right leg, subtotal body (excluding the head), head, whole body
- areal BMD at the first to fourth lumbar vertebrae and total lumbar spine

- areal BMD at the neck of femur and total hip.

Participants assessed at centres other than UHS did not undergo DXA scanning owing to the lack of standardisation and accuracy between different manufacturers. Although DXA scanning is thought to be the gold standard in body composition analysis (255, 256), additional methods were used as described above in order to estimate body composition in participants who attended research centres other than Southampton. Measurement of skinfold thickness is convenient and fast method with portable equipment required (257). BIA has been acknowledged as a practical, simple, inexpensive and non-invasive method to assess body composition (258).

3.7.7.1 Derived measurements from DXA scanning

Spine bone mineral apparent density (BMAD) was calculated for each participant according to the method described by Ward et al (2007) and age-specific SDS were calculated using reference data from the same study (254).

3.7.8 Assessment of muscle function

Muscle strength was assessed using a JAMAR hand dynamometer (JAMAR, Patterson Medical Holdings Incorporated, Sammons Preston, Rolyan, Bolingbrook, Illinois, USA) to measure grip strength in the hands according to a standardised approach (259). Participants were seated in chairs with back supports and arm rests. Owing to the different locations of research visits, the same style chair could not always be ensured. However, participants were positioned so that their knees were flexed to 90^{o} and their feet were flat and supported. When the seated position resulted in participants' feet resting above the ground, an appropriate support was placed beneath the feet. Each arm was supported in turn by the armrest of the chair. If this was not possible, a table was used to rest the forearm. Participants were offered the chance to feel dynamometer prior to measurement. The same introduction and standard encouragement was given to each participant when performing grip strength assessment. The maximal force exerted was visually assessed by the movement of the peak hold needle and recorded to the nearest 1 kilogram of force. Six measurements were taken on alternate sides therefore three results were obtained for each hand. The maximum value on each side was used to calculate age- and sex-specific SDS using normative British data (260).

3.8 Methods to study quality of life

3.8.1 Wellbeing questions

The six questions for assessment of participant wellbeing (see Appendix F) were developed by the study steering group. Although not validated, these were taken from existing questionnaires and had been assessed by an experienced epidemiologist and statistician as well as trialled in advance of the study commencing.

3.8.2 Schedule for Evaluation of Individual Quality of Life – Direct Weighting

I administered The Schedule for Evaluation of Individual Quality of Life – Direct Weighting (SEIQoL-DW) (see appendix R) (261) face-to-face during the study appointment. Using the same introduction, each participant was first asked to identify the five most important aspects of his/her current life. If participants were unable to nominate five areas, a standard list of examples was provided. Following this elicitation of the five 'cues', participants were then asked to rate their current status for each cue from 0 to 100, with 0 being 'the worst possible' and 100 being 'the best possible' situation. I developed a visual scale to facilitate this process (see Appendix S). These satisfaction scores are referred to as 'cue levels'. Finally, participants were asked to assess the way in which the different cues compared in importance to one another. This was undertaken by drawing a circle and depicting the contribution of each cue as a segment of a pie chart. In this way, each segment (cue) was assigned a percentage with the sum of all segments being 100%. Where participants found this challenging, an additional step was added: the domains were first ranked and any that were of equal importance were ranked together. Following this percentages were allocated. The scores provided for relative weighting of each cue are referred to as 'cue weights'. Difficulties in understanding, calculation or completion were recorded following administration of the SEIQoL-DW.

The SEIQoL-DW index score is calculated by first converting the expression of cue levels and cue weights from percent to decimal i.e. 70% becomes 0.70 and 95% becomes 0.95 and so on. The cue level and cue weight are then multiplied for each of the five cues and the sum of these calculations is the SEIQoL-DW index score. The scores obtained reflected participants' perceived quality of life and numerical values provide a scale for data analysis.

3.8.3 Sheehan Disability Scale

The Sheehan Disability Scale (262) (see Appendix Q) is a brief tool for respondents to report symptoms and their effects over the preceding week. This was administered face-to-face. 'Symptoms' was a term that not all participants with SRS could identify with. This was therefore

expanded and explained as 'having Russell-Silver syndrome'. The first three questions respectively enquire about disruption over the preceding week to a participant's:

1. work/volunteer activities/schoolwork
2. social life and leisure activities
3. family life and home responsibilities.

For each question above there is a ten-point visual analogue scale, which allows numerical assessment of disability. There are additional verbal descriptive anchors; 'not at all', 'mildly', 'moderately', 'markedly' and 'extremely'. The final two questions enquire about: 1) the number of days missed from work, school or activities in the home and 2) the number of days when symptoms caused reduced productivity.

3.9 Molecular genetic studies of SRS

Blood samples were obtained at study appointments and genomic DNA was extracted from peripheral blood leukocytes in the central Wessex processing facility using a chemagic Magnetic Separation Module instrument (PerkinElmer chemagen Technologie GmbH, Baesweiler, Germany). Quality control was performed on samples using 1 µl genomic DNA, using a NanoDrop ND-100 spectrophotometer (Labtech International, Ringmer, UK), to determine DNA concentration, protein levels (260/280 ratio), and RNA levels (260/230 ratio). Two techniques were then employed to investigate the imprinted regions of interest; methylation-specific polymerase chain reaction (MS-PCR) and methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA). Experiments were performed in order to evaluate methylation at the differentially methylated regions (DMRs) of the following imprinted genes: *GRB10* (7p12), *PEG1*/*MEST* (7q32), *H19* (ICR1: 11p15), *IGF2* DMR0 (11p15), *KCNQ1OT1* (ICR2: 11p15), *DLK1* (14q32), *NESPAS*/*GNAS-AS* (20q13). MS-PCR was performed by me under supervision from Professor Deborah Mackay on the samples of 17 individuals recruited to the study and on the remaining samples by Professor Mackay. MS-MLPA was performed on the samples of 17 individuals recruited to the study and this work was completed by me during my laboratory placement.

3.9.1 Methylation-specific polymerase chain reaction

MS-PCR is based on the technique of polymerase chain reaction (PCR), whereby DNA is amplified in a reaction involving template DNA, primers, deoxynucleoside triphosphates (dNTPs) and a thermostable DNA polymerase, in a buffered aqueous solution. DNA polymerase acts to extend an existing chain of DNA but not to initiate its creation. Primers are single-stranded, short sequences of nucleotides which are chemically synthesised to complement, and therefore target,

specific DNA sequences. The presence of a primer allows DNA polymerase to extend the primer sequence; a process which only occurs from the 5' to 3' direction. Each PCR reaction cycle involves three stages: 1) the temperature is first increased to 95^{o}C briefly to denature the DNA, separating the double-stranded DNA to two single strands; 2) the temperature is reduced to approximately 60^{o}C to allow hybridisation of the primers to the single-strand template DNA; 3) the temperature is increased to the optimal level (approximately 72^{o}C) for extension by thermostable DNA polymerase. In each cycle, both the original template DNA as well as the newly synthesised DNA are replicated. Therefore, as a result of using two primers and repeated cycles of denaturation, hybridisation and extension, there is an exponential increase in DNA product. Following completion of the PCR reaction, there is an excess of amplified DNA to such an extent the product is considered to be a preparation of the target sequence.

Treatment of DNA with sodium metabisulfite causes deamination of cytosine into uracil. However, 5-methylcytosine bases are resistant to deamination. Although not naturally present in DNA, uracil forms base-pairs with adenine as thymidine does. Following bisulfite treatment of the template DNA, unmethylated cytosine bases become uracil bases and methylated cytosine bases are unaltered. In the PCR product, thymidine is substituted for uracil. Therefore, bisulfite treatment transforms DNA methylation differences into DNA sequence differences (see Figure 3.3).

DNA sequence 1:

C G C 5MeC G 5MeC G A T G A T

DNA sequence following bisulfite treatment:

U G **U** 5MeC G 5MeC G A T G A T

Figure 3.3 The effects of bisulfite treatment on DNA sequence. An example of a DNA sequence including methylated cytosine bases (5MeC). Following bisulfite treatment, unmethylated cytosine bases are converted to uracil bases, however 5MeC bases are unaltered.

Methylation-specific duplex PCR was designed following the method of Zeschnigk et al (1997) to

measure the relative proportions of unmethylated and methylated alleles in DNA samples, using primers with different 3' terminal nucleotides designed to complement differentially-methylated DNA (263). Three primers were used to target each region of interest. One primer was fluorescently labelled and not selective for unmethylated or methylated DNA. Two reverse primers were used – one selective for the methylated allele; one for the unmethylated. During hybridisation with the bisulfite-treated template DNA, a primer with adenosine as the terminal nucleotide would complement uracil in the unmethylated template: guanine would complement cytosine in the methylated template. A reaction including the three primers should yield paternal and maternal products in a ratio reflecting that of the original DNA. Using a known quantity of DNA and restriction of the number of PCR cycles maintains amplification in the linear range which permits ratiometry. The non-selective forward primer is fluorescently-labelled, permitting the detection and accurate sizing of amplicons.

3.9.2 MS-PCR experiments in this study

The primer sequences are shown in Table 3.3 and the reagents and materials are shown in Table 3.4. 0.5-2 µg of genomic DNA was treated with sodium meta-bisulfite using the EZ-DNA methylation kit, according to manufacturer's instructions (Zymo Research, Orange, CA, USA), except that DNA was eluted in 50 µl per µg of starting DNA. These methods have previously been described by Poole et al (142).

Table 3.3 Differentially methylated regions (DMRs) of interest and the complementary primer sequences.

DMR	Primer sequences
GRB10 (7p12)	Me: CGGTAGGCGGGTAGGGGGTCGCGC Un: GTGAGTTTGTGGTAGGTGGGTAGGGGGTTGTGTG fam: CCYCCCYCTCTCCAAATACTCAAATAAACTCC
PEG1/*MEST* (7q32)	Me: CGGAGTGGTTGTAGTTGTTCGGCGCGGC Un: GTAGTTGTTTGGTGTGGTGTTGTTTTGTGTGGG fam: CCAACCACACCCCCTCRTTCCCACC
H19 (ICR1: 11p15)	Me: CGTTTGTTAGTAGAGTGCGTTCGCGAGTCG Un: GGTTGTTTATTGTTTGTTAGTAGAGTGTGTTTGTG fam: ATAACAGAAAAAACCCCTTCCTACCACCATCAC
IGF2 DMR0 (11p15)	Me: GTTTGACGAGGTTAGTGAGGGACGGCG Un: ATAGTTTTGTTTGATGAGGTTAGTGAGGGATGGTG fam: CCAAAACAATTTCCCTAAAAATACTCATTCATAC
KCNQ1OT1 (ICR2: 11p15)	Me: TTCGTGTTGAGGCGACGCGGCGATCGTTTTGTT Un: TGATTGTTTTTGTTGTTGTTGATGTGGTGATTG fam: CCACCTCACACCCAACCAATACCTCATA
GTL2 (14q32)	Me: CGCGTTTTGGTTCGTTGGTTTTGGCGGCG Un: GTGTAGATGGTGGAGAGTAGAGAGGGAGTGTG fam: CTCCAACAACAAAACCCAAAATCAAACAAACTCTC
GNAS-AS (20q13)	Me: GGTAGACGCGCGAGTAGGTCGCG Un: GTTTTGTTAGGTAGATGTGTGAGTAGGTTGTGG fam: CAAACRCAAAAACTCCCACTACCCCAACC

Table 3.4 Reagents and materials used for MS-PCR and MS-MLPA experiments in this study.

Reagent	Supplier
TRIS-EDTA (TE) buffer	Sigma Life Sciences (St. Louis, USA) as 100x which contains 1.0M TRIS and 0.1M EDTA. This was diluted to 1x
Molecular biology grade water (free from nucleic acids, DNAses and RNAses)	Sigma Life Sciences (St. Louis, USA)
PCR primers	Sigma-Aldrich (Poole, UK)
Deoxynucleotide triphosphates (dNTPs)	Promega (Madison, USA)
HotStarTaq DNA polymerase	Qiagen (Düsseldorf, Germany)
10X buffer	Qiagen (Düsseldorf, Germany)
HiDi formamide	Applied Biosystems (Warrington, UK)
PCR plastics	4titude (Wotton, UK)
ROX	Applied Biosystems (Warrington, UK)
$MgCl_2$	Qiagen (Düsseldorf, Germany)

Table 3.5 PCR conditions common to all reactions described in Table 3.6, Table 3.7, Table 3.8 and Table 3.9.

Reagent	Volume (µL)
dNTPs (200 µM)	1.000
10X buffer (15 mM)	1.000
genomic DNA	1.000
$MgCl_2$ (25 mM)	0.500
HotStarTaq DNA polymerase	0.075

Table 3.6 Additional PCR conditions specifically used for investigations on the DMRs of KCNQ1OT1, GRB10, IGF2 DMR0 and GTL2. Square brackets indicate the three stages of each PCR cycle with the number outside the square brackets denoting the number of cycles performed. Durations shown in minutes (min) and seconds (s). Primers: fluorescently labelled (fam), for the methylated allele (me) and for the unmethylated allele (un).

Reagent	Volume (µL)
dH_2O	5.675
fam primer (0.5 µM)	0.250
me primer (0.5 µM)	0.250
un primer (0.5 µM)	0.250
95°C 15 min [95°C for 20 s/60°C for 20 s/72°C for 20 s]$_{28}$ 72°C for 10 min followed by 15°C soak.	

Table 3.7 Additional PCR conditions specifically used for investigations on the DMR of NESPAS. Square brackets indicate the three stages of each PCR cycle with the number outside the square brackets denoting the number of cycles performed. Durations shown in minutes (min) and seconds (s). Primers: fluorescently labelled (fam), for the methylated allele (me) and for the unmethylated allele (un).

Reagent	Volume (µL)
dH_2O	5.675
fam primer	0.250
me primer	0.250
un primer	0.250
95°C 15 min [95°C for 20 s/60°C for 20 s/72°C for 20 s]$_{28}$ 72°C for 10 min then 60°C for 60 min followed by 15°C soak.	

Table 3.8 Additional PCR conditions specifically used for investigations on the DMR of MEST.

Square brackets indicate the three stages of each PCR cycle with the number outside the square brackets denoting the number of cycles performed. Durations shown in minutes (min) and seconds (s). Primers: fluorescently labelled (fam), for the methylated allele (me) and for the unmethylated allele (un).

Reagent	Volume (µL)
dH_2O	5.575
fam primer	0.250
me primer	0.500
un primer	0.100
95°C 15 min [95°C for 20 s/60°C for 20 s/72°C for 20 s]$_{28}$ 72°C for 10 min then 60°C for 60 min followed by 15°C soak.	

Table 3.9 Additional PCR conditions specifically used for investigations on the DMR of H19.

Square brackets indicate the three stages of each PCR cycle with the number outside the square brackets denoting the number of cycles performed. Durations shown in minutes (min) and seconds (s). Primers: fluorescently labelled (fam), for the methylated allele (me) and for the unmethylated allele (un).

Reagent	Volume (µL)
dH_2O	5.675
fam primer	0.250
me primer	0.250
un primer	0.250
95°C 15 min [95°C for 20 s/55°C for 20 s/72°C for 20 s]$_{28}$ 72°C for 10 min followed by 15°C soak.	

1 µl of bisulfite-treated DNA (equivalent to approximately 40 ng of input DNA) was added to 9 µl of the relevant reaction mixtures, therefore each PCR experiment was performed in a total reaction volume of 10 µl. DNA was amplified using a 2730 thermal cycler (Applied Biosystems, Warrington, UK). The reaction conditions are shown in Table 3.5, Table 3.6, Table 3.7, Table 3.8 and Table 3.9.

DNA fragments were resolved on a Genetic Analyser 3100 (Applied Biosystems, Warrington, UK). Fragments were analysed using GeneScan and Genotyper software (Applied Biosystems, Warrington, UK). The peak heights were taken from electropherograms and peak height ratios calculated to compare the methylated allele to the unmethylated. Results showing very weak (>100) or very strong (>7000) peak heights were discarded because inaccuracy in their calculations would lead to incorrect ratiometry.

It should be emphasised that (a) this method reflects only the proportion of methylated versus unmethylated DNA in the sample analysed, and somatic mosaicism is not addressed; (b) the method is semi-quantitative; (c) the method does not directly discriminate primary methylation disturbance from copy number change or uniparental disomy, such that when methylation disturbance is detected, secondary analysis is employed where possible to discriminate the underlying cause.

Secondary analysis may include microsatellite analysis (to confirm or exclude uniparental disomy), or copy number variation analysis by MS-MLPA. Microsatellite analysis was not performed as part of this research, however participants underwent molecular diagnostic testing prior to recruitment to STAARS therefore microsatellite analysis had been performed where appropriate.

3.9.3 Methylation-specific multiplex ligation-dependent probe amplification

Following denaturation of sample genomic DNA, in standard multiplex ligation-dependent probe amplification (MLPA), probes consisting of two separate oligonucleotides are added to the test sample. Each oligonucleotide contains a primer sequence and hybridises to a unique, target DNA sequence. The oligonucleotide probes hybridise immediately adjacent to one another and are ligated by a specific ligase enzyme. Because each oligonucleotide probe contains a primer sequence, in a PCR reaction, only ligated probes would be amplified in an exponential manner. Multiple targets can be investigated simultaneously because the oligonucleotides are highly specific and the same primer pair is used for each probe (264). Up to 55 targets can be evaluated in a single reaction and copy number changes can be detected as well as methylation specific analysis (265).

MS-MLPA does not require prior bisulfite treatment but utilises a methylation-sensitive restriction endonuclease, HhaI, to differentially permit ligation depending on the methylation status of the target sequence. MS-MLPA probes are synthesised to contain a HhaI restriction site within the target sequence. HhaI will cleave the hybrid of probe and sample DNA if the site is not methylated. However, HhaI is unable to cleave if the target DNA is methylated, in which case the fragment will then be amplified exponentially during PCR (266) (Figure 3.4).

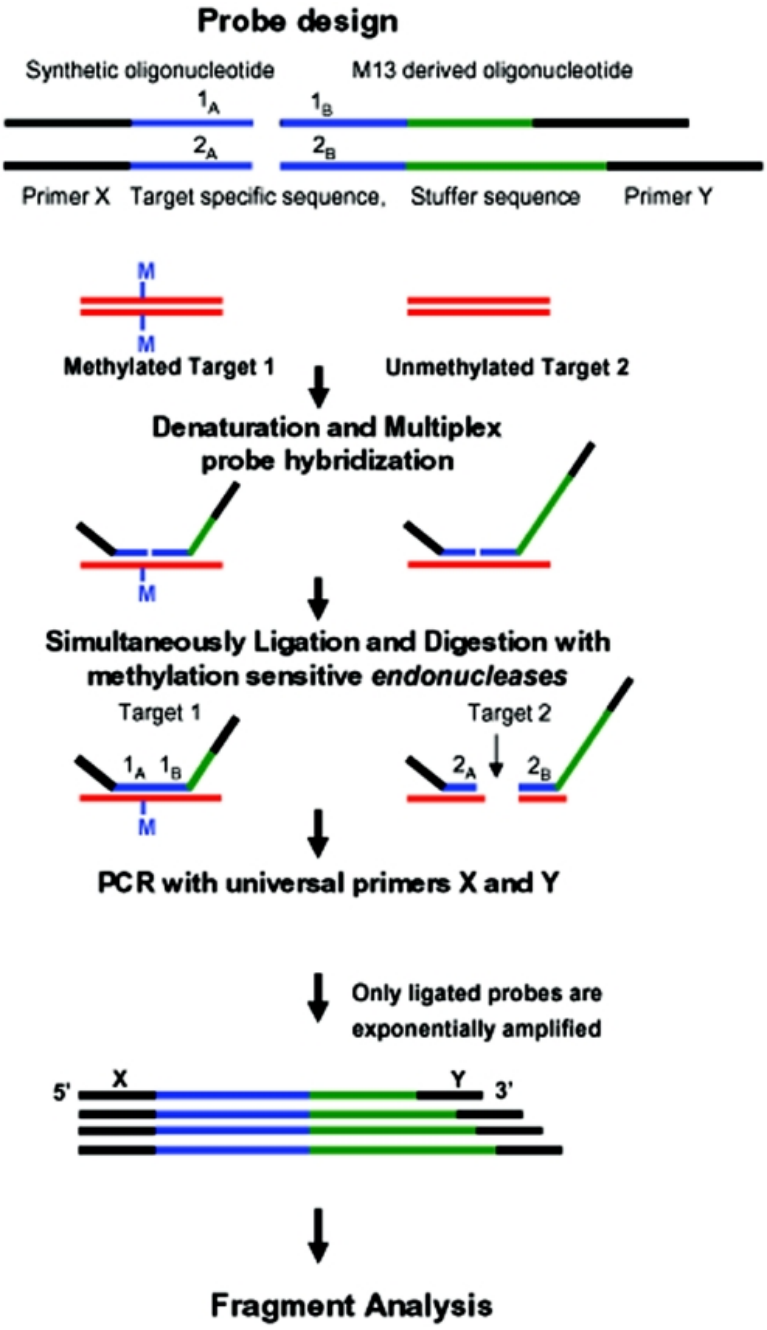


Figure 3.4 Methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA). Reproduced with permission from (266).

Part of the reaction is digested by HhaI and ligation occurs simultaneously (as shown in Figure 3.4) and part is treated as standard MLPA (ligation only).

Each non-digested probe has a unique ligation product of a specific length. Two peak patterns are produced for each sample: one from the digestion and ligation reaction – for methylation profiling; and one from the ligation only reaction – for copy number analysis. Methylation percentage is calculated by comparing the peak patterns of HhaI-treated and HhaI-untreated reactions. The methylation profile of a test sample is evaluated by comparison of the probe methylation percentages in that test sample to the percentages of reference samples.

3.9.4 MS-MLPA experiments in this study

MS-MLPA was performed using kits (SALSA MLPA ME030 and SALSA MLPA ME032) from MRC Holland and according to the manufacturer's instructions (MRC-Holland BV, Amsterdam, Netherlands; version 33; 29-11-2012). HhaI was obtained from New England Biolabs UK (Hitchin, Hertfordshire, UK).

3.10 Data collection and management

Data management processes were reviewed during the NHS Research and Development application process. Research files were stored in the Wessex Clinical Genetics Service offices in the Princess Anne Hospital. This department is locked outside normal working hours. In accordance with NHS Research and Development protocols research notes will be stored for 15 years from the research study end date.

The 'Doctor-Managed Record of History and Examination of RSS Proband' was completed during and immediately following each research study appointment. Original copies were photocopied for data entry. I obscured participants' names, highlighted relevant areas within text fields for data entry and labelled the forms with study identification numbers. Data entry using these redacted photocopies was performed by staff at the MRC Lifecourse Epidemiology Unit, University of Southampton, Southampton General Hospital. The anonymised data was entered using Microsoft Access and a sample of data from the Microsoft Access database was reviewed against the photocopied forms and any discrepancies reviewed.

The database was converted to an SPSS file and transferred using an encrypted USB flash drive. Following data entry, the redacted photocopies were retained in the Wessex Clinical Genetics Service.

Data from the 'Doctor-Managed Record of History and Examination of RSS Proband' of five participants was subsequently entered directly into the SPSS database by me. All previous data entered was reviewed by me and required corrections made.

Where parents attended the study appointment, the 'Early Medical History Questionnaire for Parent of STAARS Participant' was completed during the study appointment or posted in advance to be completed beforehand and brought to the appointment. Where parents did not attend the study appointment the form was posted separately to the participant, directly to the parent(s) (with permission), or given to the participant at the study appointment. All data entry from original copies of the 'Early Medical History Questionnaire for Parent of STAARS Participant' was performed by me.

All digital patient-identifiable data were stored on a Microsoft Excel spreadsheet saved on a NHS computer network within a designated area for the Wessex Clinical Genetics Service. These data were managed by me and accessed by other members of the research team.

The following information was requested from study participants: the hospitals in which they had previously received treatment, the names of their treating physicians and for their written consent to request their medical records. The UHS CRN research team contacted these hospitals and requested copies of medical records. Electronic and hard copy medical records were received and stored in the Wessex Clinical Genetics Service. Medical records were reviewed by me, Professor Temple and Dr Davies and the following data was extracted from medical records:

- Birth details: gestation, weight, length, occipto-frontal circumference
- Growth measurements: height, weight, decimal age if available, date of measurement
- Details of growth hormone treatment: doses, dates of commencement/ending/dose change, dosage per kg or m^2 where available
- Pubertal staging and date
- Details of treatment to delay the onset of puberty: medication(s), doses, associated dates
- Parental height values (not usually clarified whether these were measurements or reported heights)

Data was obtained from clinical notes entries and/or letters following appointments. The date used was that of the clinic rather than the letter where possible. Where multiple measurements were available, preference was given to Paediatric Endocrine clinics. Data was only obtained from growth charts if there was no alternative. Where two measurements were available in cases of asymmetry, measurements were included for the longer side. In cases where there had been discontinuous growth hormone treatment, data was used from the start of first treatment and

end of final treatment and discontinuous treatment was noted. Height data for the start and end of treatment was accepted if within six months of the treatment dates.

In cases, where the initial review of medical records was insufficient, I reviewed my research notes to ascertain whether treatment had been reported at other hospitals and then further notes were requested. In one case where the hospital denied the individual had been a patient there, I contacted the participant to re-confirm hospital was correct and to ascertain her maiden name. With the new surname her records were found. Four out of five participants who were still receiving growth hormone at the time of the study appointment had completed this treatment at the time their medical notes were reviewed. In these cases, their height, weight and BMI, the calculated SDS were taken from the subsequent medical notes.

This data was first recorded using MS Word. I then transferred the typed data into an Excel spreadsheet and converted units from imperial to metric for lengths/heights and weights. Within the spreadsheet, I calculated decimal ages, converted growth hormone doses from IU to mg and calculated the growth hormone dosages. I used the LMS growth add-in to calculate standard deviation scores for height, weight, body mass index and occipito-frontal circumference. The Excel spreadsheet was converted to an SPSS database.

3.11 Methods to establish research collaboration

Through my supervisor, I was introduced to and became a member of the European Network of Human Congenital Imprinting Disorders (EUCID) which was established with support from a Collaboration in Science and Technology (COST) grant (BMBS COST Action BM1208). The EUCID network aimed to gather together researchers working on eight disorders of imprinting including SRS. Researchers included medical doctors and scientists from France, Germany, Italy, The Netherlands, Poland, Slovenia, Spain and the UK. Four working groups (WG) were formed to focus on different aspects of research; WG1 – clinical diagnosis; WG2 – molecular diagnosis; WG3 – guidelines; WG4 – dissemination.

3.11.1 Short term scientific mission

I successfully applied for a EUCID short-term scientific mission and in May 2014 attended both the Patient-Expert SRS convention in Paris and two specialist SRS clinics with Professor Irène Netchine (AP-HP, Hôpitaux Universitaires Paris Est (AP-HP) Hôpital des Enfants Armand Trousseau, Service d'Explorations Fonctionnelles Endocriniennes, Paris, France) and Dr Madeleine Harbison (Mount Sinai School of Medicine, New York, USA).

The aim of my STSM was to learn about diagnosis and specialist management of SRS from world experts. The objectives were to attend lectures and clinics to learn about clinical features, genotype-phenotype correlations and multi-disciplinary team management. I hoped that by learning from the vast experience in Paris, my understanding of SRS would be improved, which would then help to refine my research questions.

I attended the SRS convention in Paris and witnessed patient and family testimonies regarding living with SRS. There were lectures from experts in the management of nutrition, feeding, oral desensitisation, gastroenterology, neurology, orthopaedics, and orthodontics. These lectures gave an insight into the common problems and approach to specialist treatment.

I attended specialist clinics where patients with SRS were seen and assessed. I summarised patient history questionnaires and growth measurements before the appointments. During the appointments, I was involved with history taking and examination of patients. I was responsible for recording the medical details for each patient and producing patient reports for review by both senior doctors.

15 patients attended: these included six with loss of methylation of ICR1 on chromosome 11p15.5 and four with maternal uniparental disomy for chromosome 7. I learned about treatment protocols implemented at Hospital Trousseau, Paris – including nutritional management to optimise growth without promoting excessive intake. Within this protocol the aim is for patients to maintain weights within 70-80% of that ideal for their height. I also learned about the theory of avoidance of insulin resistance with the aim of avoiding potential early adrenarche. I learned about the utility of monitoring for the development of ketonuria as a herald for hypoglycaemia. Other treatment aspects I learned about included oral desensitisation therapy and the orthopaedic management of leg length discrepancy. It was useful to hear subjective reports from doctors, patients and their families about observed differences in body composition, muscle strength, and endurance with growth hormone treatment.

3.11.2 Collaboration

I presented my research plan, interim updates regarding recruitment and challenges to recruitment in this rare disease. As the study progressed, the steering group identified that achieving adequate recruitment would be difficult. Both Professor Temple and I discussed potential collaboration with clinicians at centres within EUCID. Professor Irène Netchine (AP-HP, Hôpitaux Universitaires Paris Est (AP-HP) Hôpital des Enfants Armand Trousseau, Service d'Explorations Fonctionnelles Endocriniennes, Paris, France) and Professor Gerhard Binder (University Children's Hospital, Pediatric Endocrinology, Tuebingen, Germany) agreed to

collaborate in this research by sharing anonymised data on individuals with molecularly confirmed SRS. I proposed the minimum set of data to be collected about patients from collaborating centres:

- Molecular diagnosis
- Birth data – gestation, weight and SDS, length and SDS, head circumference and SDS
- At most recent follow-up – age (in months and years), height and SDS, weight, BMI and SDS
- Growth hormone treatment – yes or no
- If treated with GH, age, height and SDS at start and at end of growth hormone treatment and dosing regimen
- If untreated with GH, age, height and SDS at approximately age 2-5 years
- Age at onset of puberty
- Additional treatment(s) – GnRH analogues, aromatase inhibitors and accompanied by details of age, height and SDS at start and end of treatment.

I received the above data in the form of two Excel spreadsheets. I then amalgamated the data from the STAARS UK cohort, the French cohort and the German cohort into one Excel spreadsheet. All data was anonymised if not already done before transfer. I ensured consistency of formatting for data including calendar dates, decimal points rather than commas. I converted growth hormone doses so that the unit used within the data was consistent. Where there was a range of growth hormone doses, I used the mean average value. In cases where there had been discontinuous growth hormone treatment, data was used from the start of first treatment and end of final treatment. Discontinuous treatment was noted. Height data for the start and end of treatment was accepted if within six months of the treatment dates. Treatment durations were provided by collaborating researchers. In three cases the duration was noted as a minimum or approximate values. These data were excluded. The absolute values were reviewed for outlying SDS and then checked with the collaborating research teams. Errors in SDS were corrected where necessary. I aim to publish the results from this research collaboration regarding the effects of GH treatment on final height and body mass index in SRS.

3.11.3 Silver-Russell syndrome international consensus statement

I was involved in research to refine the clinical diagnostic criteria for SRS and agree a consensus on the clinical diagnosis, molecular testing and management of SRS. I performed the PubMed literature search along with another member of the EUCID network: we each searched using the terms ‘Silver Russell syndrome’ and ‘Russell Silver syndrome’ and compared the 516 articles that were identified. I was involved in the compilation of relevant research articles.

All abstracts found were divided between the consensus committee members to be reviewed and suitable publications allocated to the relevant working group. Those relating to clinical and molecular diagnosis of SRS as well as specialist management were included and any irrelevant abstracts were excluded.

At a pre-consensus meeting in April 2015, funding, structure, methodology, accommodation and travel logistics were discussed. The consensus meeting was held over three days in October 2015. The 41 consensus participants included clinical geneticists, molecular geneticists, paediatric endocrinologists, a gastroenterologist and five representatives from parent support groups. Amongst the consensus participants, there were designated representatives from the European Society for Pediatric Endocrinology, the Pediatric Endocrine Society, the Asia Pacific Pediatric Endocrine Society, and Sociedad Latino-Americana de Endocrinología Pediátrica [the Latin American Society for Pediatric Endocrinology].

I was a member of working group 3, which was tasked with considering clinical management of SRS and I had the role of joint secretary. Prior to the consensus, the chairs and secretaries of each working group reviewed the articles allocated following the abstract review process. I was involved in summarising articles and formulating recommendations to be presented for discussion amongst the full working group. During the consensus meeting, recommendations were discussed in plenary sessions and revised as required. The concluding step was that of voting on the recommendations follows:

A. Evidence or general agreement allow full agreement with the recommendation

B. Evidence or general agreement are in favour of the recommendation

C. Evidence or general agreement are weak for the recommendation

D. There is not enough evidence or general agreement to agree with the recommendation

The strength of the recommendation was recorded as follows, depending on the percentage of votes received:

+ 26–49% of the votes ++ 50–69% of the votes +++ ≥70% of the votes.

3.12 Data management and statistical analysis

Data from the doctor-managed questionnaires in the UK cohort were initially entered into a database using SPSS version 21. These data were associated with the study identification numbers without participants' names. Data from the questionnaires for the parents of affected

individuals was also entered into the SPSS database. Data extracted from medical records were entered using Microsoft Word then converted into an Excel spreadsheet. All imperial measurements were converted to metric values using standard conversions.

There were some variables where discrepancies were noted between the information from the participant, parent(s) and/or medical records. These included: gestation at birth, birth weight, and parental heights. There was no overlap in data obtained for birth length and birth occipito-frontal circumference therefore this problem did not arise. Data from medical notes was given preference to recalled data. Where there were numerous values in the medical notes, the mean value was used.

SDS were calculated for the following using the LMS growth Excel add-in and UK 1990 data (267): occipito-frontal circumference for age, height for age, weight for age, and body mass index for age. Participants' ages were calculated to two decimal places. Birth parameter SDS were adjusted for gestational age using gestation in weeks and days. The upper age limits of the reference data for occipito-frontal circumference are 17 years and 18 years in females and males respectively. The upper age limit for height and weight is 23 years. Participants' SDS were calculated using their data for their actual age if this was available within the reference data. Where the participant was older than the upper age limit, the SDS was calculated using the data for the highest age possible. Data from the European collaboration was received in an Excel spreadsheet. SDS were calculated by the French and German researchers using population-specific data; Sempe et al in the former and Niklasson and Prader in the latter case.

Variables were examined for normality of distribution. Categorical variables were analysed using Chi square tests or the Fisher's exact test where an expected cell count was less than five. Continuous variables following a normal distribution were analysed using two-sample t-tests or linear regression as appropriate. Continuous variables with non-normal distributions were analysed using the Mann-Whitney U test for comparison of two categories and the Kruskal-Wallis test for three or more. Comparison of ordinal variables between two groups was performed using the Mann-Whitney U test. Data analysis was performed using SPSS version 24.

P values <0.05 were initially considered significant. However, over recent years, there has been discussion regarding the application of statistical testing to research and some resultant misinterpretations of P values (268-270). A recent comment article with over 800 signatories advocated that a P value greater than a threshold such as 0.05 should not comprise the sole contribution to decisions or inferences about associations, effects or differences (i.e. they alone should not lead to a conclusion that there is 'no association' or 'no difference'). The authors also advised against stating a result is 'statistically significant' and proposed that studies should not be

reported to conflict if one demonstrates a statistically significant result and another does not. The authors highlight that P values continue to have utility but should not be applied in a dichotomous or categorical manner (270). It has been suggested that a more refined aim of statistical analysis is to provide an assessment of certainty or uncertainty regarding the size of an effect (269). Lack of statistical significance does not indicate a small effect size and particularly in a study with small numbers, large effects may be 'drowned in noise' and fail to be detected as statistically significant (269). This final point is especially relevant to some of the studies presented in this thesis owing to the small numbers in the sample size. Therefore, in the results presented, P values approaching 0.05 may be referred to as 'suggesting' differences or effects, and results with P values >0.05 may be discussed.

Chapter 4 Study recruitment of the UK STAARS cohort, molecular genetic investigations and participant demographics

4.1 Recruitment

The study commenced in April 2014 and was completed in March 2018. 40 participants were recruited to the study.

Figure 4.1 demonstrates the routes of recruitment. Initial contacts were made directly to individuals with SRS where they had given permission to be re-contacted as a result of previous research ethics agreements. Alternatively, the treating doctor was contacted. Members of the Child Growth Foundation were sent study information by Mrs Jenny Child, (CGF Membership secretary, Birmingham, UK).

One potential participant, identified from the IDFOW study, died in an accident after the initial contact. The reasons for exclusion of the three individuals from the WRGL route were: 1) identification via another route; 2) family living abroad; 3) inability to ascertain contact details for the treating physician. Following the expression of interest by individuals with SRS, five people were excluded who had negative molecular genetic testing. Despite initially expressing an interest in the study, three individuals (a parent in one case) could neither be contacted by telephone nor in writing. One further individual, identified via the CGF, expressed an interest in the study but subsequently was unable to proceed owing to childcare commitments.

Overall, 120 study information packs were distributed and 40 participants were recruited. This represents an uptake of 33.3%. The most effective route of recruitment was via the IDFOW study because 10 participants were recruited from 18 possible individuals. The highest number of participants were recruited via the CGF (17 from a possible 36 individuals). The least effective route of recruitment was via ascertaining individuals tested at the WRGL and contacting the referring physicians.

The numbers of participants recruited in each month of the study are shown in Figure 4.2 and the cumulative recruitment is shown in Figure 4.3.

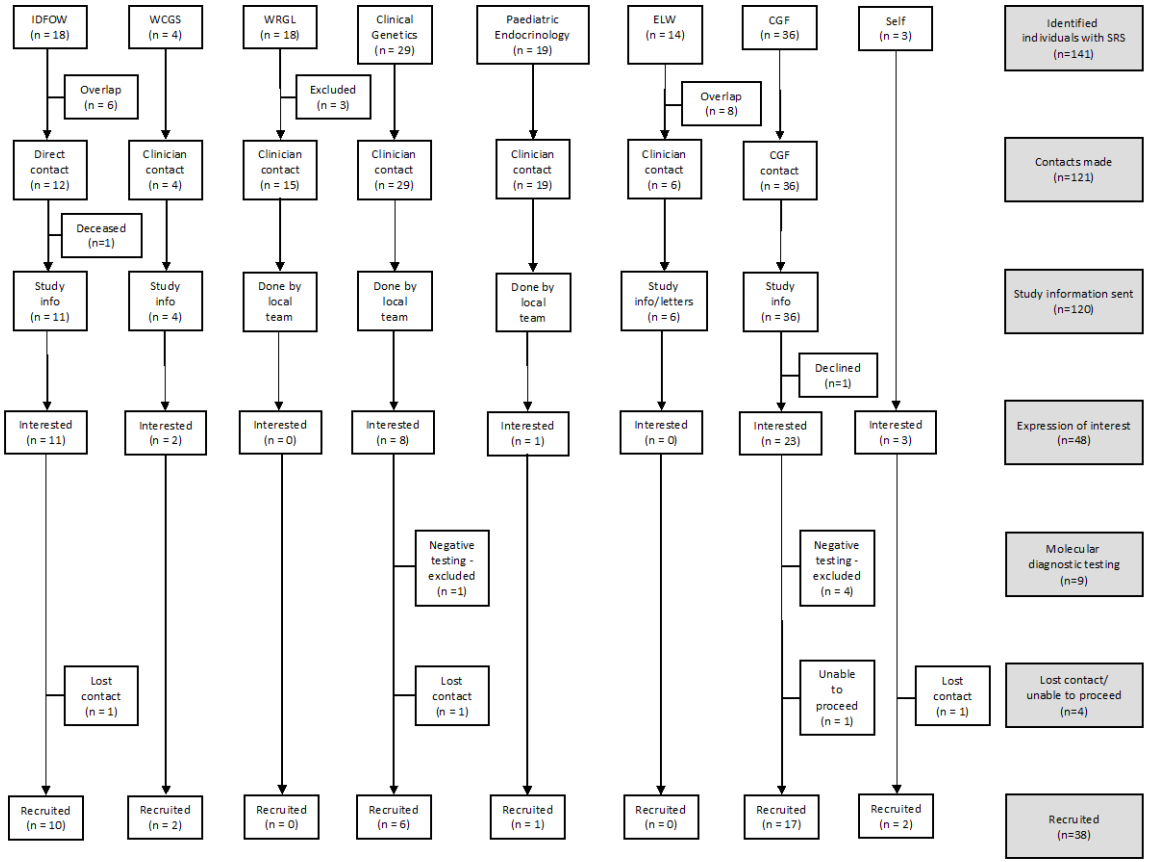


Figure 4.1 Sources of identification of individuals for recruitment to the study. n = number. 'Imprinting Disorders–finding out why' study (IDFOW), the Wessex Clinical Genetics Service (WCGS), the Wessex Regional Genetics Laboratory (WRGL), a collaborating researcher, Dr Emma Wakeling (ELW) and the Child Growth Foundation (CGF). Boxes labelled 'overlap' indicate individuals identified via more than a single route.

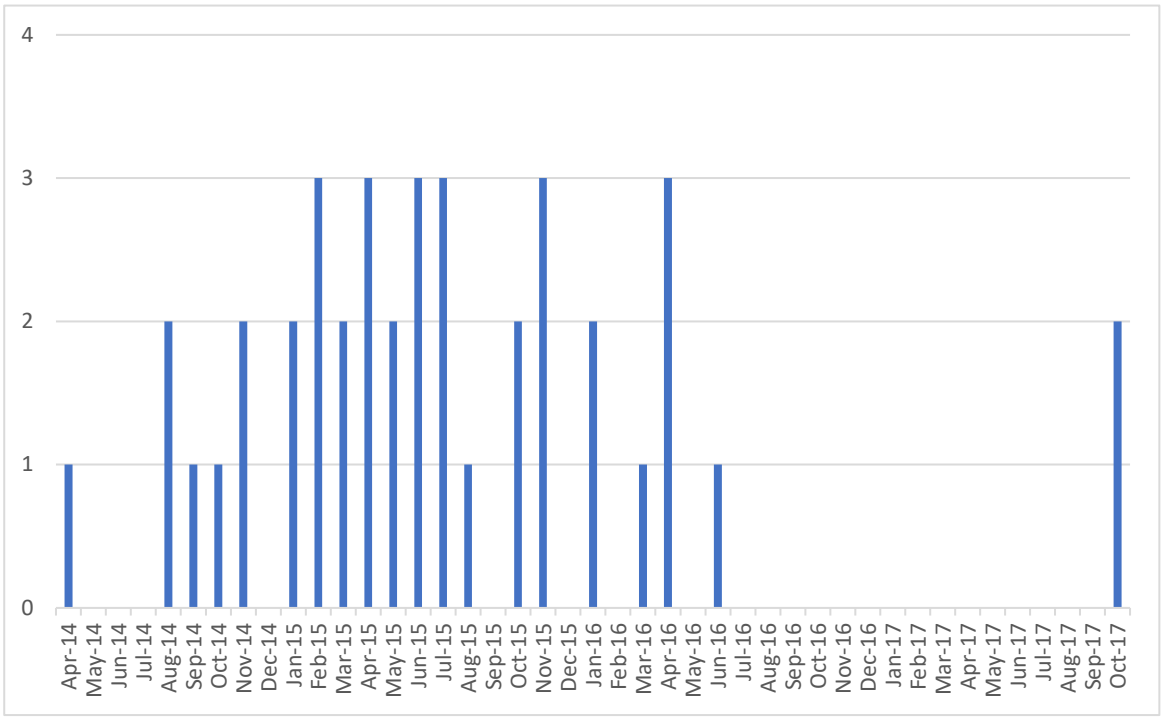


Figure 4.2 Participant recruitment (number) by calendar month and year of the study.

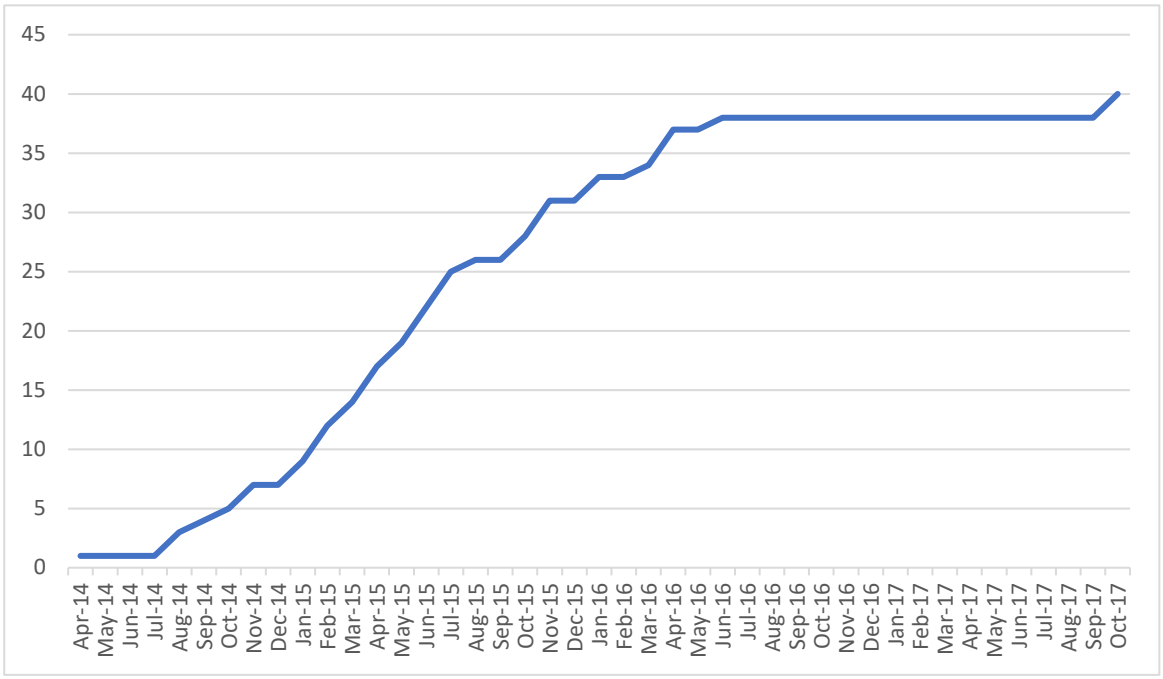


Figure 4.3 Cumulative participant recruitment (number) by calendar month and year of the study.

Table 4.1 Participant demographics of the UK STAARS cohort. Gender and growth hormone treatment presented as number (n) and column percentage. Birth and growth parameters presented as median and interquartile range. * indicates full ranges where quartiles could not be calculated. Number shown where data incomplete. ^ indicates row percentages. P-values calculated between the subgroups of the cohort.

	Whole cohort	H19/IGF2 LOM	matUPD7	matUPD14	No methylation change	P value
n	40	27 (67.5)^	6 (15.0)^	3 (7.5)^	4 (10.0)^	
Gender						
Male (n, %)	17 (42.5)	12 (44.4)	3 (50)	1 (33.3)	1 (25.0)	0.894
Female (n, %)	23 (57.5)	15 (55.6)	3 (50)	2 (66.7)	3 (75.0)	
Age, years (median, range)	27.90 (13.32-69.71)	32.35 (13.32-69.71)	19.74 (14.47-33.93)	13.53-28.52	20.02 (19.69-28.86)	0.088
Birth parameters						
Gestation at birth, weeks	38.0 (37-40) (n=38)	39 (37.0-40.6) (n=25)	38.0 (35.1-38.1)	38.00 (37.00-39.29)*	39 (35.0-41.5)	0.379
Birth weight, g	1870 (1485-2262) (n=40)	1760 (1458-2098)	1805 (1505-2513)	2187 (1870-2665)*	2305 (1460-2966)	
Birth weight SDS	-2.84 (-3.97 to -2.55) (n=39)	-3.54 (-4.20 to -2.64) (n=26)	-2.19 (-2.98 to -1.29)	-2.41 (-2.59 to -1.39)*	-2.20 (-3.18 to -0.58)	**0.024**
Birth length, cm	41.8 (39.9-45.5) (n=14)	40.6 (39.9-47.3) (n=10)	43.0 (n=1)*	45.0 (n=1)*	40.8 (37.0-44.5) (n=2)*	
Birth length SDS	-3.78 (-4.98 to -1.10) (n=13)	-4.06 (-5.26 to -0.55) (n=9)	-3.05 (n=1)*	-1.53 (n=1)*	-3.90 (-4.74 to -3.06) (n=2)*	0.788
Birth head circumference, cm	32.5 (30.7-34.6) (n=14)	33.8 (32.0-35.4) (n=8)	27.0 (n=1)*	31.8 (31.6-32.6) (n=2)*	31.3 (27.0-34.0) (n=3)*	
Birth head circumference SDS	-0.72 (-1.63 to -0.36) (n=14)	-0.56 (-1.33 to 0.29) (n=8)	-0.79 (n=1)*	-1.25 (-1.86 to -0.63) (n=2)*	-1.35 (-3.06 to -1.25) (n=3)*	0.287
Growth parameters						
Height, cm	151.5 (141.1-158.8)	153.0 (143.5-160.9)	156.8 (145.7-160.7)	141.3 (141.0-149.0)*	145.3 (136.5-154.7)	
Height SDS	-2.91 (-3.99 to -1.21)	-3.13 (-3.87 to -1.02)	-2.19 (-3.03 to -1.32)	-3.79 (-4.28 to -2.21)*	-3.80 (-4.50 to -2.15)	0.5
Weight, kg	47.9 (41.5-58.7)	45.65 (38.90-62.30)	52.05 (45.38-56.81)	60.90 (40.35-81.30)*	50.40 (35.25-56.25)	
Weight SDS	-1.29 (-3.43 to -0.15)	-1.83 (-4.66 to -0.11)	-1.47 (-2.17 to -0.14)	-0.85 (-1.36 to 2.14)*	-2.00 (-4.80 to -0.24)	0.691
BMI, kg/m^2	20.8 (18.0-27.0)	19.7 (17.5-28.0)	22.9 (17.6-25.0)	27.4 (20.2-40.91)*	21.8 (17.5-27.8)	
BMI SDS	-0.48 (-1.65 to 1.31)	-0.80 (-1.99 to 1.49)	0.07 (-1.34 to 1.08)	1.33 (0.49-3.53)*	-0.17 (-1.99 to 1.56)	0.313
Growth hormone treatment						
Yes	27 (67.5)	17 (63.0)	6 (100)	0	4 (100)	**0.008**
No	13 (32.5)	10 (37.0)	0	3 (100)	0	

Table 4.2 Clinical characteristics of individuals aged <18 years and ≥18 years. P-values calculated for comparison between individuals aged <18 years and individuals aged ≥18years. Gender, molecular diagnosis, and growth hormone treatment presented as number (n) and percentage. Age presented as median (full range). Growth parameters presented as median (interquartile range, IQR).

	Whole cohort	Individuals <18 years	Individuals ≥ 18 years	P value
n	40	9	31	
Gender				
Male (n, %)	17 (42.5)	3 (33.3)	14 (45.2)	0.707
Female (n, %)	23 (57.5)	6 (66.7)	17 (54.8)	
Age, years (median, range)	27.90 (13.32-69.71)	13.83 (13.32-17.44)	31.28 (19.69-69.71)	
Molecular genetic diagnosis (n, %)				
H19/IGF2 LOM	27 (67.5)	5 (55.6)	21 (67.7)	
matUPD7	6 (15.0)	3 (33.3)	3 (9.7)	0.231
matUPD14	3 (7.5)	1 (11.1)	2 (6.5)	
No methylation change	4 (10.0)	0 (0)	4 (12.9)	
Growth parameters (median, IQR)				
Height, cm	151.5 (141.1-158.8)	154.0 (138.1-161.7)	150.1 (141.0-157.1)	0.849
Height SDS	-2.91 (-3.99 to -1.21)	-1.51 (-3.37 to -0.60)	-3.17 (-4.03 to -1.82)	0.055
Weight, kg	47.9 (41.5-58.7)	42.00 (33.95-47.88)	55.45 (44.10-62.30)	
Weight SDS	-1.29 (-3.43 to -0.15)	-1.20 (-3.52 to -0.27)	-1.72 (-3.49 to -0.15)	1.000
BMI, kg/m^2	20.8 (18.0-27.0)	17.5 (16.2-19.9)	21.9 (19.3-28.0)	
BMI SDS	-0.48 (-1.65-1.31)	-1.33 (-1.69 to 0.30)	-0.38 (-1.67 to 1.56)	0.248
Growth hormone treatment (n, %)				
Yes	27 (67.5)	8 (88.9)	19 (61.3)	0.226
No	13 (32.5)	1 (11.1)	12 (38.7)	

4.2 Results of molecular genetic investigations

Figure 4.4 shows electropherograms from the STAARS molecular genetic analysis. For each sample, methylated/unmethylated peak height ratios were calculated and then normalised to control values. The results for all individuals recruited are shown in Table 4.3.

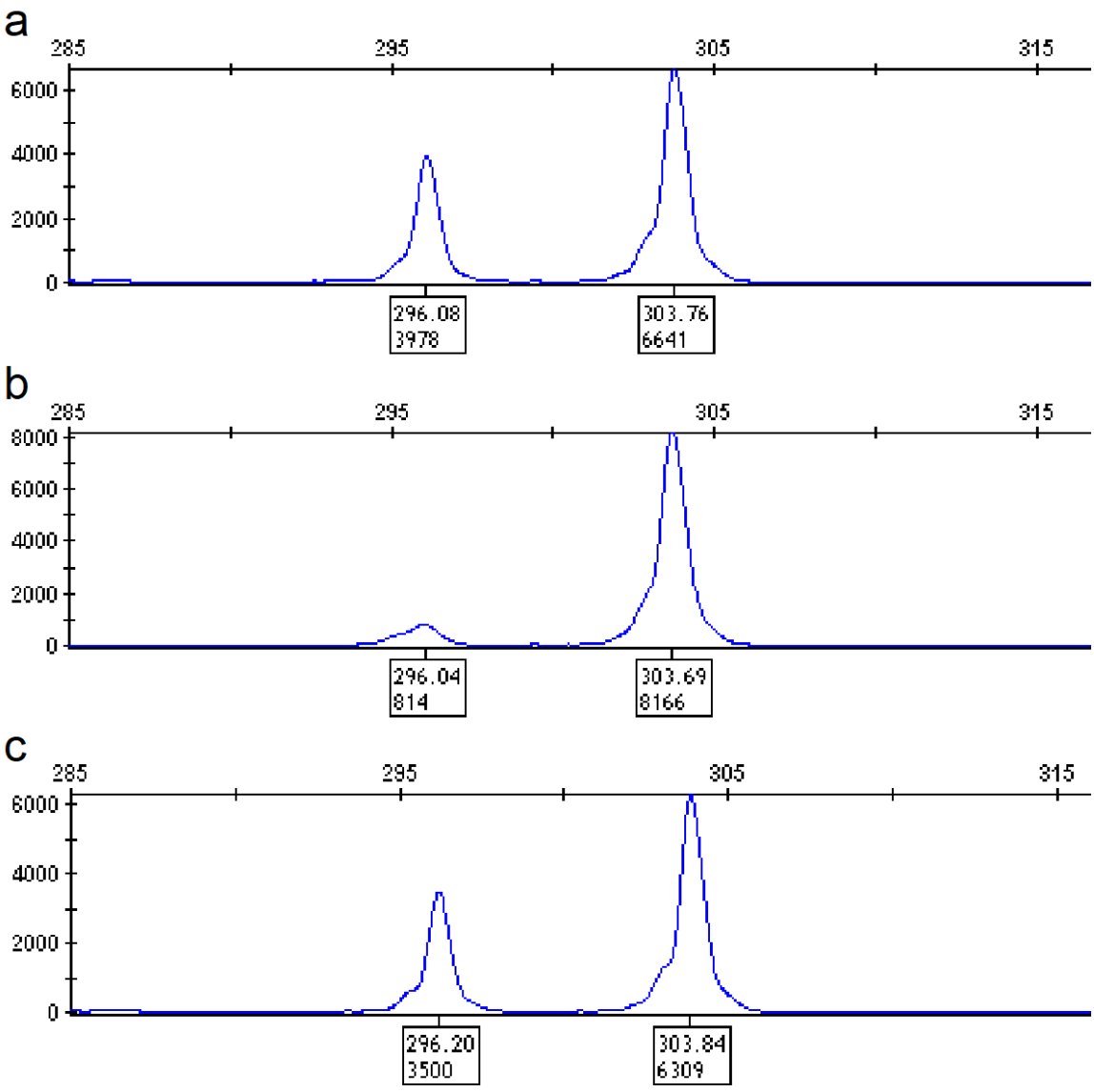


Figure 4.4 Electropherograms from molecular genetic testing. These show methylated peaks on the left and unmethylated peaks on the right. Panel a depicts the result in a case of matUPD7 from the UK cohort (Case ID 26). Panel b depicts the result in a case of H19IGF2 LOM from the UK cohort (Case ID 35). Panel c depicts the result from a normal control.

Table 4.3 Results from molecular testing of the UK STAARS cohort. The DMRs tested were KCNQ1OT1, H19, IGF2, GRB10, PEG1/MEST, DLK1 and NESPAS/GNAS. Researcher who performed the tests indicated; Oluwakemi Lokulo-Sodipe (OLS) or Professor Deborah Mackay (DJGM)

Case ID	Molecular testing method	KCNQ1OT1/H19/IGF2	GRB10	PEG1/MEST	DLK1	NESPAS/GNAS	Researcher
1	MS-PCR and MS-MLPA	LOM at H19 and DMR0	normal	normal	normal	normal	DJGM, OLS
2	MS-PCR and MS-MLPA	LOM at H19 and DMR0	normal	normal	normal	normal	DJGM, OLS
3	MS-PCR	normal	complete hypermethylation	complete hypermethylation	normal	normal	DJGM
4	MS-PCR and MS-MLPA	LOM at H19 and DMR0	normal	normal	normal	normal	DJGM, OLS
5	MS-PCR and MS-MLPA	LOM at H19 and DMR0	normal	normal	normal	normal	DJGM, OLS
6	MS-PCR and MS-MLPA	LOM at H19 and DMR0	normal	normal	normal	normal	DJGM, OLS
7	MS-PCR	normal	normal	normal	normal	normal	DJGM
8	MS-PCR and MS-MLPA	LOM at H19 and DMR0	normal	normal	normal	normal	DJGM, OLS

Case ID	Molecular testing method	KCNQ1OT1/H19/IGF2	GRB10	PEG1/MEST	DLK1	NESPAS/GNAS	Researcher
9	MS-PCR and MS-MLPA	LOM at H19 and DMR0	normal	normal	normal	normal	DJGM, OLS
10	MS-PCR and MS-MLPA	LOM at H19 and DMR0	normal	normal	normal	normal	DJGM, OLS
11	MS-PCR	normal	complete hypermethylation	complete hypermethylation	normal	normal	DJGM
12	MS-PCR	normal	normal	normal	normal	normal	DJGM
13	MS-PCR and MS-MLPA	LOM at H19 and IGF20	normal	normal	normal	normal	DJGM, OLS
14	MS-PCR and MS-MLPA	LOM at H19 and IGF20	normal	normal	normal	normal	DJGM, OLS
15	MS-PCR	normal	normal	normal	normal	normal	DJGM
16	MS-PCR	normal	complete hypermethylation	complete hypermethylation	normal	normal	DJGM
17	MS-PCR and MS-MLPA	LOM at H19 and IGF20	normal	normal	normal	normal	DJGM, OLS

Case ID	Molecular testing method	KCNQ1OT1/H19/IGF2	GRB10	PEG1/MEST	DLK1	NESPAS/GNAS	Researcher
18	MS-PCR	normal	complete hypermethylation	complete hypermethylation	normal	normal	DJGM
19	MS-PCR and MS-MLPA	LOM at H19 and IGF20	normal	normal	normal	normal	DJGM, OLS
20	MS-PCR	normal	normal	normal	complete LOM	normal	DJGM
21	MS-PCR and MS-MLPA	normal	normal	normal	complete LOM	normal	DJGM, OLS
22	MS-PCR and MS-MLPA	LOM at H19 and IGF20	normal	normal	normal	normal	DJGM, OLS
23	MS-PCR and MS-MLPA	normal	complete hypermethylation	complete hypermethylation	normal	normal	DJGM, OLS
24	MS-PCR and MS-MLPA	LOM at H19 and IGF20, duplication of maternal origin	normal	normal	normal	normal	DJGM, OLS

Case ID	Molecular testing method	KCNQ1OT1/H19/IGF2	GRB10	PEG1/MEST	DLK1	NESPAS/GNAS	Researcher
25	MS-PCR and MS-MLPA	LOM at H19 and IGF20	normal	normal	normal	normal	DJGM, OLS
26	MS-PCR and MS-MLPA	normal	complete hypermethylation	complete hypermethylation	normal	normal	DJGM, OLS
27	MS-PCR	LOM at H19 and IGF20	normal	normal	normal	normal	DJGM
29	MS-PCR	LOM at H19 and IGF20	normal	normal	normal	normal	DJGM
30	MS-PCR	normal	complete hypermethylation	complete hypermethylation	normal	normal	DJGM
31	MS-PCR	LOM at H19 and IGF20	normal	normal	normal	normal	DJGM
32	MS-PCR	LOM at H19 and IGF20	normal	normal	normal	normal	DJGM
33	MS-PCR	LOM at H19 and IGF20	normal	normal	normal	normal	DJGM
34	MS-PCR	LOM at H19 and IGF20	normal	normal	normal	normal	DJGM

Case ID	Molecular testing method	KCNQ1OT1/H19/IGF2	GRB10	PEG1/MEST	DLK1	NESPAS/GNAS	Researcher
35	MS-PCR and MS-MLPA	LOM at H19 and IGF20	normal	normal	normal	normal	DJGM, OLS
36	MS-PCR and MS-MLPA	LOM at H19 and IGF20	normal	normal	normal	normal	DJGM, OLS
37	MS-PCR and MS-MLPA	LOM at H19 and IGF20	normal	normal	normal	normal	DJGM, OLS
38	MS-PCR and MS-MLPA	LOM at H19 and IGF20	normal	normal	normal	normal	DJGM, OLS
39	MS-PCR and MS-MLPA	normal	normal	normal	normal	normal	DJGM, OLS
40	MS-PCR and MS-MLPA	LOM at H19 and IGF20	normal	normal	normal	normal	DJGM, OLS
41	MS-PCR and MS-MLPA	LOM at H19 and IGF20	normal	normal	normal	normal	DJGM, OLS

4.3 Recruitment via European COST collaboration

Recruitment to the UK STAARS cohort presented challenges (see section 4.1). The target of 100 participants was not achieved within the UK alone. After forming collaborations through the European COST network for imprinting disorders and working on the first international consensus for the diagnosis and management of Silver-Russell syndrome, a research collaboration was established with Professor Irène Netchine, Hôpital d'Enfants Armand Trousseau, Paris, France and Professor Gerhard Binder, University Children's Hospital, Tübingen, Germany. Using the methods described in section 3.11.2, data on individuals with genetically confirmed SRS was obtained. The French cohort, identified by Professor Irène Netchine, included 17 individuals and the German cohort, identified by Professor Gerhard Binder, included 21 individuals. The demographics of overall STAARS cohort of 71 participants and study results are discussed in Chapter 6.

4.4 Participant demographics

4.4.1 Overall UK cohort and subgroups

This chapter presents molecular diagnoses, growth at birth, height, weight, BMI, and details of growth hormone treatment in order to give an overview of the UK cohort and compare to previously published SRS cohorts. Further data and the results of detailed phenotyping will be presented in Chapter 5. The study demographics are shown in Table 4.1. The UK cohort included 40 participants aged 13.32 to 69.71 years with a median age of 27.90 years. The cohort was comprised of 17 (44.7%) male and 21 (55.3%) female individuals. The cohort was stratified on the basis of molecular genetic diagnosis; H19/IGF2 LOM was found in 27 (67.5%); matUPD7 in 6 (15.0%); and matUPD14 in 3 (7.5%). 4 (10.0%) individuals had no molecular confirmation but had been clinically diagnosed with SRS. The median birth weight was 1870 g (n=40) with median birth weight SDS of -2.84 (n=39). The median birth length was 41.8 cm (n=14) with birth length SDS of -3.78 (n=13). The median head circumference at birth was 32.5 cm (n=14) with a median SDS of -0.72 (n=14). SDS were not available for all measurements because the reference data (267) does not include very preterm infants.

Comparing the molecular subgroups, there was no statistically significant difference in: the proportions of males and females; gestation at birth, birth length parameters, head circumference at birth parameters; or any of the growth parameters. There were significant differences in birth weight SDS (p=0.024) with the lowest birth weights in the H19/IGF2 LOM group. Differences in BMI were not significant although the lowest BMI SDS were seen in the H19/IGF2 LOM and higher

BMI SDS were seen in the matUPD14 cases. The numbers of GH-treated and GH-untreated individuals were significantly different between the molecular subgroups (p=0.008) and no participant with matUPD14 had been treated with GH. The relevant GH licence would be SGA birth weight without catch-up growth. However, in matUPD14 greater birth weights might mean the SGA criterion is not met, which could explain the reduced frequency of GH treatment in this group.

Table 4.2 presents the clinical characteristics of the cohort and subgroups of individuals <18 years and those ≥18 years. The majority of individuals were aged ≥18 years (31/40). There was no significant difference in the proportions of males and females. There was a slightly higher proportion of H19/IGF2 LOM cases in those aged ≥18 years (66.7% vs 55.6%, p=0.231). Median height SDS was -1.51 (IQR -3.37 to -0.60) in individuals <18 years and -3.17 (-4.03 to -1.82) in individuals aged ≥18 years (p=0.055). The age subgroups were not significantly different in weight SDS or BMI SDS.

4.4.2 Clinical characteristics of participants with H19/IGF2 LOM

Table 4.4 delineates the clinical characteristics of individuals with H19/IGF2 LOM. This group included one male and four females aged <18 years. There were 11 males and 11 females aged ≥18 years. There were no significant differences in the growth parameters between these four groups.

The median ages of adult males and adult females was similar (32.88 years and 31.29 years respectively). Males ≥18 years had a median final height of 156.9 cm (IQR 150.3-171.3) with a median height SDS of -3.13 (IQR -4.09 to -1.02). Females ≥18 years had a median final height of 144.7 cm (IQR 141.0-157.1) and a median height SDS of -3.17 (IQR -3.79 to -1.12).

Males and females ≥18 years had similar median weights (55.50 kg and 54.65 kg respectively) which corresponded to SDS of -2.22 in males and -0.47 in females. BMI was higher in adult females compared to adult males (median 22.3 kg/m^2; median SDS -0.02 compared to 19.7 kg/m^2 and SDS -1.58). BMI SDS across the four subgroups was not significantly different (p=0.201).

4.4.3 Clinical characteristics of participants with maternal UPD 7

Table 4.5 shows the clinical characteristics of the six individuals with matUPD7. Three were aged <18 years with a median age of 15.92 years in males and 16.89 years in the single female. Three individuals aged ≥18 years included one male aged 22.03 years and two females who had a

median age of 29.52 years. The median final heights in individuals aged ≥18 years with matUPD7 was 159.3 cm (SDS -2.69) in the single male and 143.6 cm (SDS -3.35) in the females.

Table 4.4 Clinical characteristics of individuals with H19/IGF2 LOM.

H19/IGF2 LOM	**All**	**Males <18 years**	**Females <18 years**	**Males ≥18 years**	**Females ≥18 years**
n	27	1	4	11	11
Age, years (median, range)	32.35 (13.32-69.71)	13.83	13.79 (13.32-15.40)	32.88 (23.44-69.71)	31.29 (24.40-56.85)
Birth parameters (median, range)					
Gestation at birth, weeks	39 (37.0-40.6) (n=25)	42	36.9 (33.9-39.6)	38.29 (37.0-40.0)	40.6 (37.1-41.7) (n=9)
Birth weight, g	1760 (1458-2098)	2650	1459 (1163-1768)	1871 (1560-2070)	1760 (1304-2200)
Birth weight SDS	-3.54 (-4.20 to -2.64) (n=26)	-2.66	-3.46 (-4.15 to -2.83)	-3.70 (-4.20 to -2.59)	-3.64 (-4.31 to -2.53) (n=10)
Birth length, cm	40.6 (39.9-47.3) (n=10)	n/a	n/a	42.1 (40.0-48.7) (n=6)	40.6 (38.6-45.4) (n=4)
Birth length SDS	-4.06 (-5.26 to -0.55) (n=9)	n/a	n/a	-3.95 (-5.35 to -0.31) (n=6)	-4.06 (-5.31 to -0.43)* (n=3)
Birth head circumference, cm	33.8 (32.0-35.4) (n=8)	n/a	30.5 (28.0-30.5)* (n=2)	34.5 (32.0-35.3) (n=5)	35.6 (n=1)*
Birth head circumference SDS	-0.56 (-1.33 to 0.29) (n=8)	n/a	-1.35 (-2.05 to -0.65)* (n=2)	-0.55 (-1.06 to 0.27) (n=5)	0.63 (n=1)*
Growth parameters (median, range)					
Height, cm	153.0 (143.5-160.9)	164	144.0 (128.9-153.7)	156.9 (150.3-171.3)	144.7 (141.0-157.1)
Height SDS	-3.13 (-3.87 to -1.02)	0.34	-2.69 (-4.78 to -0.54)	-3.13 (-4.09 to -1.02)	-3.17 (-3.79 to -1.12)
Weight, kg	45.65 (38.90-62.30)	47.10	33.95 (24.38-43.86)	55.50 (41.30-65.85)	54.65 (44.25-64.53)
Weight SDS	-1.83 (-4.66 to -0.11)	-0.11	-2.98 (-5.16 to -0.62)	-2.22 (-5.45 to -0.68)	-0.47 (-2.13 to 0.70)
BMI, kg/m^2	19.7 (17.5-28.0)	17.5	16.2 (14.61-18.69)	19.7 (18.4-28.0)	22.3 (19.7-29.1)
BMI SDS	-0.80 (-1.99 to 1.49)	-0.53	-1.66 (-2.73 to -0.26)	-1.58 (-2.33 to 1.49)	-0.02 (-1.09 to 1.84)
Growth hormone treatment (n)					
Yes	17 (63.0)	1	4	8	4
No	10 (37.0)	0	0	3	7

Table 4.5 Clinical characteristics of individuals with matUPD7.Data presented as median (range) unless indicated with * which indicates incomplete data available.

matUPD7	**All matUPD7**	**Males <18 years**	**Females <18 years**	**Males ≥18 years**	**Females ≥18 years**
n	6	2	1	1	2
Age, years (median, range)	19.74 (14.47-33.93)	15.96 (14.47-17.44)*	16.89	22.03	29.52
Birth parameters (median, range)					
Gestation at birth, weeks	38.0 (35.1-38.1)	38.3 (38.0-38.6)*	36.86*	38.0*	34.0 (30.0-38.0)*
Birth weight, g	1805 (1505-2513)	2590 (2435-2745)*	1710*	1644*	1494 (1088-1899)*
Birth weight SDS	-2.19 (-2.98 to -1.29)	-1.37 (-1.60 to -1.13)*	-2.80 *	-3.53*	-2.06 (-2.78 to -1.34)*
Birth length, cm	43.0 (n=1)*	n/a	n/a	n/a	43.0 (n=1)*
Birth length SDS	-3.05 (n=1)*	n/a	n/a	n/a	-3.05 (n=1)*
Birth head circumference, cm	27.0 (n=1)*	n/a	n/a	n/a	27.0 (n=1)*
Birth head circumference SDS	-0.79 (n=1)*	n/a	n/a	n/a	-0.79 (n=1)*
Growth parameters (median, range)					
Height, cm	156.8 (145.7-160.7)	162.0 (159.4-164.6)*	154.4	159.3	143.6 (139.50-147.7)*
Height SDS	-2.19 (-3.03 to -1.32)	-1.23 (-1.70 to -0.76)*	-1.51	-2.69	-3.35 (-4.03 to -2.67)*
Weight, kg	52.05 (45.38-56.81)	45.33 (42.00-48.65)*	58.95	55.45	51.30 (46.50-56.10)*
Weight SDS	-1.47 (-2.17 to -0.14)	-1.75 (-2.28 to -1.22)*	0.27	-2.13	-1.00 (-1.72 to -0.28)*
BMI, kg/m^2	22.9 (17.6-25.0)	17.3 (16.5-18.0)*	24.7	21.9	24.8 (23.9-25.7)*
BMI SDS	0.07 (-1.34 to 1.08)	-1.36 (-1.38 to -1.33)*	1.23	-0.38	0.78 (-0.53 to 1.04)*
Growth hormone treatment (n)					
Yes	6	2	1	1	2
No	0	0	0	0	0

Table 4.6 Clinical characteristics of individuals with matUPD14. Standard deviation scores (SDS) calculated for measurements including body mass index (BMI). * indicates incomplete data available.

matUPD14	All	Female <18 years	Male ≥18 years	Female ≥18 years
n	3	1	1	1
Age, years	24.65 (13.53-28.52)	13.53	24.65	28.52
Birth parameters				
Gestation at birth, weeks	38.00 (37.00-39.29)*	39.29	38	37
Birth weight, g	2187 (1870-2665)*	2665	2025	1870
Birth weight SDS	-2.41 (-2.59 to -1.39)*	-1.39	-2.59	-2.41
Birth length, cm	45.0 (n=1)*	n/a	n/a	45.0
Birth length SDS	-1.53 (n=1)*	n/a	n/a	-1.53
Birth head circumference, cm	31.8 (31.6-32.6) (n=2)*	n/a	31.6	32.0
Birth head circumference SDS	-1.25 (-1.86 to -0.63) (n=2)*	n/a	-1.86	-0.63
Growth parameters				
Height, cm	141.3 (141.0-149.0)*	141.3	149.0	141.0
Height SDS	-3.79 (-4.28 to -2.21)*	-2.21	-4.28	-3.79
Weight, kg	60.90 (40.35-81.30)*	40.35	60.90	81.30
Weight SDS	-0.85 (-1.36 to 2.14)*	-0.85	-1.36	2.14
BMI, kg/m^2	27.4 (20.2-40.91)*	20.2	27.43	40.9
BMI SDS	1.33 (0.49-3.53)*	0.49	1.33	3.53
Growth hormone treatment (n)				
Yes	0	0	0	0
No	3	1	1	1

Table 4.7 Clinical characteristics of individuals with no methylation change. Standard deviation scores (SDS) calculated for measurements including body mass index (BMI).

No methylation change	All	Male ≥18 years	Females ≥18 years
n	4	1	3
Age, years	20.02 (19.69-28.86)	19.69	20.31 (19.73-28.86)
Birth parameters			
Gestation at birth, weeks	39 (35.0-41.5)	38	40 (34.0-42.0)*
Birth weight, g	2305 (1460-2966)	3175	2270 (1149-2340)*
Birth weight SDS	-2.20 (-3.18 to -0.58)	0.11	-3.06 (-3.22 to -2.64)*
Birth length, cm	40.8 (37.0-44.5) (n=2)*	n/a	40.8 (37.0-44.5) (n=2)*
Birth length SDS	-3.90 (-4.74 to -3.06) (n=2)*	n/a	-3.90 (-4.74 to -3.06) (n=2)*
Birth head circumference, cm	31.3 (27.0-34.0) (n=3)*	n/a	33.0 (27.0-34.0)*
Birth head circumference SDS	-1.35 (-3.06 to -1.25) (n=3)*	n/a	-1.35 (-3.06 to -1.25) (n=2)*
Growth parameters			
Height, cm	145.3 (136.5-154.7)	155.4	137.1 (136.3-152.7)*
Height SDS	-3.80 (-4.50 to -2.15)	-3.15	-4.44 (-4.53 to -1.82)*
Weight, kg	50.40 (35.25-56.25)	46.5	54.30 (31.50-56.90)*
Weight SDS	-2.00 (-4.80 to -0.24)	-3.49	-0.52 (-5.23 to -0.15)*
BMI, kg/m^2	21.8 (17.5-27.8)	19.3	24.4 (16.96-28.91)*
BMI SDS	-0.17 (-1.99 to 1.56)	-1.19	0.85 (-2.26-1.80)*
Growth hormone treatment (n)			
Yes	4	1	3
No	0	0	0

4.4.4 Clinical characteristics of participants with maternal uniparental disomy for chromosome 14

The characteristics of three individuals with matUPD14 are shown in Table 4.6. The heights were 141.3 (SDS -2.21) in the female aged <18 years, 149.0 cm (SDS -4.28) in the male ≥18 years and 141.0 cm (SDS -3.79) in the female ≥18 years. Weights ranged from 40.35 kg to 81.30 kg with SDS of -0.85 in the female aged <18 years and 2.14 in the female aged ≥18 years. BMI ranged from 20.2 kg/m^2 (SDS 0.49) to 40.9 kg/m^2 (SDS 3.53). GH treatment was not received in any case. Owing to the small sample number (n=3 total; n=1 in each group) significance testing was not performed.

4.4.5 Clinical characteristics of participants with clinically diagnosed SRS

Table 4.7 shows the clinical characteristics of the four individuals aged ≥18 years with a clinical diagnosis of SRS but no methylation change on molecular genetic testing. The median birth weight was 2305 g (IQR -3.18 to -0.58) with SDS -2.20 (IQR -3.18 to -0.58). The median height at study appointment was 145.3 cm (IQR 136.5-154.7) with SDS of -3.80 (IQR -4.50 to -2.15). The median weight was 50.49 kg (SDS -2.00) and median BMI was 21.8 (SDS -0.17).

4.4.6 Treatment with recombinant growth hormone

Overall 27 (67.5%) participants had previously received GH treatment and 13 (32.5%) were untreated (Table 4.1). In the subgroup aged <18 years 88.9% (8/9) individuals had received GH compared to 61.3% (19/31) in those aged ≥18 years (Table 4.2).

There were significant differences in GH treatment across the subgroups (p=0.008) (Table 4.1). In the H19/IGF2 LOM subgroup 63.0% (17/27) (Table 4.4) were GH-treated compared to 100% (6/6) in the matUPD7 subgroup (Table 4.5). No individuals with matUPD14 had been treated (Table 4.6) with GH and all those with a clinical diagnosis of SRS had received GH treatment (Table 4.7). Further evaluation of GH treatment and its impact will be discussed in Chapter 6, Chapter 7 and Chapter 8.

4.5 Discussion

4.5.1 Recruitment of a UK cohort with SRS

There are many impediments to rare disease research and recruitment contributes greatly to the challenges. In contrast to other research, it was not possible to ascertain individuals from hospital

clinics as there are no clinics dedicated to adults with SRS. Although medical problems in adulthood have been described in adults with SRS, cohorts of adults have not been examined for long-term outcomes therefore the potential need for secondary care clinics has not been established and there are no transition clinics in the UK. There was no consensus on clinical management prior to 2016. National Commissioning Group (formerly known as the National Specialist Commissioning Advisory Group) funding had not been allocated for SRS clinics, and affected individuals generally do not have a physician overseeing their care. Further, there is no UK registry of individuals with SRS. Furthermore, individuals with SRS are typically discharged to their primary care physician at final height, making identification of adults with SRS even more challenging.

Improvements to research infrastructure, such as registries and research networks, have been suggested as these might promote efficient and successful collaboration and patient care in rare disease (271). A national registry for SRS could potentially store contact information on individuals who have expressed an interest in being contacted about clinical research for which they would be eligible. Several participants in this research would have been candidates for such a registry.

Multiple routes of recruitment were implemented in an attempt to maximise recruitment to this study. Even so, these methods relied on individuals who were diagnosed clinically and/or with molecular genetic tests. It is likely that older adults with short stature may not have received such a diagnosis as the tests were not widely available until recently. The recruitment uptake of 33.3% (40 participants recruited after 120 study information packs sent out) is an estimate as some participants became aware of the study via digital methods including social media and the STAARS website. Nevertheless, this uptake appears low and several factors may have influenced this. There may have been a perceived lack of medical problems, lack of time to commit to a research appointment, work and family commitments and concern about issues that might be uncovered. In addition, several adults had not previously undergone genetic testing and were not recruited following negative testing in advance of the study appointment (n=5). Recruitment from IDFOW may have been more successful as these individuals had already shown motivation to be involved in clinical research and they may have had positive experiences of research that encouraged them to engage further. Membership of the CGF demonstrates a willingness to engage with the SRS community and potentially a desire to learn more about the condition. This method of recruitment may have been more effective as individuals were keen to find out more about SRS and the research was supported by questions from members and their families.

The routes that were least successful were contacting genetics services and clinicians (ascertained from Wessex Regional Genetics Laboratory and through Paediatric Endocrinology departments). This may reflect the process involved: letters were written from the research team with the aim of the receiving team then sending on the study information. This method also required time and effort from the referring physicians, who would undoubtedly have had other pressures on their time. Discussions about the research study allowed for more in-depth explanation as well as questions to be addressed whereas affected individuals who were contacted by post may not have regarded the research as important.

The pattern of recruitment illustrated in Figure 4.2 demonstrates a significant gap between the majority of recruitment and the final two participants. This reflects a period of leave during which recruitment was discontinued. However, the overall duration of the study was unaffected by this leave and the final two participants were included as they approached the research team. Fewer participants appear to have been recruited over holiday periods such as Christmas, New Year and summer holidays. This may reflect increased personal commitments of potential participants over those periods or reduced desire to leave from work.

Despite the limitations to recruitment, the cohort of 40 individuals with SRS included here is comparable in size to the only other previous study of adults (46). The individuals recruited to the UK STAARS cohort do not overlap with those described in that study.

UK and US reports on policy and strategy for research in rare diseases have highlighted that an integrated approach to research is required (272, 273). The paucity of robust epidemiological, clinical and health economics data as well as the spread of information across care providers and patient organisations and lack of longitudinal health outcome data lead to insufficient estimates about health and social burden (272). Although the natural history of many rare diseases is still unknown (272), learning about the disease course over time could be important for the development of therapies (273) which might have wider application (272). Further research is needed into effective management to stop individuals affected by the same rare disease receiving different care (272). The UK Department of Health report acknowledged the importance of ethics and research governance, however proposed that regulation should be proportionate to make research projects more cost-effective, accelerate research permissions and reduce the time taken to embark on research (272). The NIHR UK Rare Genetic Disease Research Consortium Agreement was developed to address these issues. At the time of this research study its implementation was in the early stages. With increased use, the processes are likely to have developed and improved further.

Proposals to improve rare disease research include:

- collaborative goals (273)
- international research collaboration (272)
- patient and public involvement (272, 273)
- application of advances in technology and science that could make research faster, easier and less expensive (273)
- the use of social media (e.g. Twitter, Facebook and PatientsLikeMe) (272)
- increased staff with expertise on rare disease research (272)
- co-ordination of patients, relatives, the NHS, UK research community and its funders (272).

Many of the above points were implemented in this research study, such as collaboration and patient and public involvement. In further work, technological advances and digital platforms, such as social media, could be implemented.

4.5.2 Demographics of the UK STAARS cohort

The cohort shows no important gender preponderance similar to other studies (20, 21, 24, 26, 32, 35, 46, 182, 274).♦ There was a higher proportion of cases of H19/IGF2 LOM than matUPD7 as would be expected from the literature. However, in the UK STAARS cohort there were 67.5% H19/IGF2 LOM compared to 30-60% in previous reports (24, 46, 182, 274). Individuals with matUPD7 comprised 15.0% of the UK STAARS cohort compared to 5-17.2% in other cohorts (24, 46, 182). Individuals with matUPD14 were included here and comprised 7.5% of the cohort. This molecular diagnosis and its inclusion in this study are discussed further below. Clinical SRS cases contributed 10.0% of cases in this study compared to 31-41% in previous cohorts (24, 46, 182). The difference in molecular genetic diagnoses may be affected by ascertainment methods in the different studies. The majority of participants recruited to STAARS underwent molecular genetic confirmation before recruitment therefore the proportion of clinical SRS diagnoses would be lower than in other studies. This approach reduced heterogeneity in the cohort.

The birth weight SDS of -2.84 seen in this cohort was similar to previous studies which have ranged from -2.79 to -3.50 (24, 46, 182, 274). The birth length SDS of -3.78 was also comparable to other cohorts reporting -3.01 to -4.13 (24, 46, 182, 274). The head circumference at birth SDS of -0.72 is within the range of previous studies of -1.5 to -0.62 (24)}(182). This shows that the

♦ N.B. The SRS cohort reported in Binder et al 2013 is not included in the analysis of the UK STAARS cohort reported in this chapter.

birth parameters observed in the UK STAARS cohort are representative of SRS and provides confidence with generalising the results of this study to other individuals with SRS. The results from birth measurements also show that birth weight and birth length remain useful clinical features for the diagnosis of SRS if data are available.

This cohort included 31 adults (i.e. individuals aged ≥18 years) and 9 individuals aged <18 years. As discussed in Chapter 3, participants aged between 13 and 18 years were included in order to increase the sample size. These two subgroups were analysed both together and separately. Combined analysis is permitted by the use of SDS for parameters for which reference data is available. While combined analysis provides greater power, analysis of growth in those aged <18 years requires different consideration. For example, absolute BMI is useful in adults and the recommended range is 20-25 kg/m^2. However, in those aged <18 years, absolute BMI varies with age and is only interpreted using standard deviation scores.

Comparisons between those aged <18 years and ≥18 years may be affected by the different molecular genetic diagnoses in the groups, which were 55.6% and 67.7% respectively for H19/IGF2 LOM and 33.3% and 45.2% matUPD7 respectively. Although GH treatment in the subgroup aged <18 years was 88.9% compared to 61.3% in the adult group, this was not statistically significant (p=0.226). The effects of GH hormone treatment might not only depend on treatment status at the time of the research appointment but also on duration of time elapsed since treatment discontinuation. Weight (42.00 kg vs 55.45 kg p=0.034) but not weight SDS (-1.20 vs. -1.72 p=1.000) was lower in the <18 year olds compared to the adults

The final height SDS of -3.17 n individuals aged ≥18 years in this study is comparable to -3.25 SDS described in a subset of cases from a previous study where 2 were GH-treated and seven were untreated (20). The same study found a weight SDS of -2.75 in those 7 cases, compared to -1.72 in the present study.

The mean height in adulthood demonstrated in this cohort is similar to that of -3.13 SDS found in the GH-untreated group in a previous study and lower than -2.12 found in the GH-treated group in the same study (46) and -2.17 described in another study of GH-treated SRS cases (182).

The clinical syndrome associated with aberrations in the imprinted locus on chromosome 14q32, is named Temple syndrome (TS) (discussed in section 1.12.1). Although this molecular diagnosis has not consistently been included in SRS cohorts, the clinical features overlap – particularly low birth weight, postnatal growth restriction leading to short stature, hypotonia and early puberty (134). MatUPD14 has been detected in 1 of 127 cases with clinical features suggestive of SRS (142) and in 2 of 85 (135) and 1 of 26 (32) clinically diagnosed SRS cases. Two case reports

describe the overlap in diagnosis; one in which a patient with Temple syndrome was 'misdiagnosed' with SRS (275) and the other describing a patient with Temple syndrome who meets diagnostic SRS criteria (276). Disruption of the 14q32.2 imprinted region has been proposed as an alternative molecular diagnosis of SRS (277). Whether or not TS and SRS are within a spectrum of the same disorder remains unclear and controversial. The adult phenotypes associated with the molecular genetic subgroups will be described, compared and contrasted in Chapter 5.

Comparison between the UK STAARS subgroups stratified by molecular genetic diagnosis is affected by the small numbers of cases in some groups which is a limitation to the evaluation of (epi)genotype-phenotype correlations in this cohort. There were significant differences in birth weight SDS between the molecular subgroups, with H19/IGF2 LOM having the lowest birth weight (-3.54 SDS vs -2.19 in matUPD7 vs -2.41 in matUPD14, P=0.024). Significant differences in growth hormone treatment between molecular genetic subgroups were also observed; GH treatment had been given in 63.0% in H19/IGF2 LOM cases; 100% in matUPD7; 0% in matUPD14 and 100% in clinical SRS. Individuals with matUPD7 may have been shorter at presentation as matUPD7 has been more frequently been associated with a height at presentation of $\leq$ -2 SDS than H19/IGF2 LOM (21) which could reflect the reason for a higher GH treatment rate in this subgroup. There are multiple reasons which might explain why individuals with matUPD14 were not treated with GH: firstly, there is no established precedent for treatment; secondly, the molecular diagnosis may have been late or perceived as an explanation for short stature rather than an indication for treatment, which is the case in other syndromes associated with short stature such as Down syndrome; thirdly, although two of the three individuals with matUPD14 were born SGA, it is possible there was a degree of catch-up growth by age 4 but early puberty resulting in short stature. A secular trend in GH treatment is probable since GH treatment is now more widely available than when first introduced. The eldest individual in the cohort, aged 69.71 years had not received GH but the next two eldest individuals, aged 54.70 and 56.85 years, had received GH treatment. The dosages of GH may be affected by changes in practice over time. Owing to the low number of individuals who were ineligible for treatment as a result of age (only one), the secular change in treatment prevalence should not affect the results present in this thesis.

Finally, it is important to note that there may be overlap between the present cohort and previous UK cohorts owing to the involvement of the researchers and institutions (e.g. Great Ormond Street Hospital) with an ongoing interest in SRS and contacting individuals who had previously been involved research.

4.5.3 Conclusions

The STAARS research study implemented many of the recommended strategies for recruitment of rare disease for research, including a high level of patient and public involvement. However, many of the challenges of identifying affected individuals with a rare disease such as SRS, continued to exist. Whilst the UK STAARS cohort is representative of SRS, there were three cases of matUPD14 and four clinical SRS cases included which may affect the interpretation and generalisability of the study findings. Therefore, the following chapters will consider H19/IGF2 LOM and matUPD7 as molecularly confirmed cases. MatUPD14 and clinical SRS cases will be analysed and discussed separately.

Chapter 5 The evolving phenotype and health outcomes of Silver-Russell syndrome – from childhood to adulthood

5.1 Introduction

The clinical features of SRS are well described during childhood (21, 32). Whether or not the phenotype changes with increasing age is unknown. This chapter will describe the UK STAARS cohort only. This cohort consists of 40 individuals (23 female; 17 male). The median age was 27.90 years; 31 were aged ≥18 years and 9 were aged <18 years. The results of the molecular genetic investigations were described in section 4.2. Molecular diagnoses included 27 individuals with H19/IGF2 LOM, 6 individuals with matUPD7, 4 individuals with clinical SRS and 3 individuals with matUPD14. In section 5.2 the clinical features in early life of the 33 individuals with H19/IGF2 LOM and matUPD7 will be presented. The clinical features in later life will be presented in section 5.3. The cases of H19/IGF2 LOM and matUPD7 in the UK cohort will be initially considered together and epigenotype-phenotype correlations will be drawn where possible. Secondly, phenotypic differences between the sexes will be described. Thirdly, differences between adolescents and adults will be discussed. Differences between clinical SRS and matUPD14 cases will be discussed in sections 5.7 and 5.8.

5.2 Clinical features of SRS in early life

5.2.1 Conception, pregnancy and growth at birth

The median maternal age at the birth of the study participant was 30.52 years (IQR 26.24-32.99, n=25); the median paternal age was 32.49 years (IQR 28.96-35.26, n=27). There was a history of infertility before the pregnancy involving the proband in 18.8% (6/32). Of these six cases, there was one case of difficulty conceiving for one year but a spontaneous pregnancy before a planned fertility clinic appointment; a spontaneous pregnancy after a first appointment at a fertility clinic; one case of two years difficulty conceiving but then a spontaneous pregnancy; one case of difficulty conceiving for two years then maternal hypothyroidism diagnosed and spontaneous pregnancy two months after treatment commenced; one case of in vitro fertilisation; and one case of in vitro fertilisation with intra-cytoplasmic sperm injection and a donor egg. There were six additional cases where there was a history of infertility in another pregnancy or pregnancy loss

bringing the total proportion of parents of cases affected by infertility or pregnancy loss to 38.7% (12/31). These additional six cases involved: two siblings whose mother had experienced two miscarriages and one still birth; one history of one miscarriage; one case where Clomid had been used in a pregnancy before the proband (but no assistance in pregnancy with proband); one history of pregnancy loss due to ectopic pregnancy; and one history of recurrent miscarriages (three) where the mother was affected with a clotting disorder. There were no significant differences in maternal age (p=0.113) or paternal age (p=0.527) between cases where there was infertility in the proband pregnancy compared to those where there was not. There were no significant differences in maternal age (p=0.508) or paternal age (p=0.979) between cases where there was any history of infertility or pregnancy loss. Overall, the rate of assisted reproductive technology in the proband pregnancies was 6.45% (2/31).

Preterm births occurred in 22.6% (7/31) of this cohort. Three were stated to have been elective Caesarean sections; three were stated to have been emergency Caesarean sections; and it was not stated in one case. The indications for Caesarean sections were unclear.

Birth weight, length and head circumference and respective SDS were presented in Chapter 4. Patterns of growth and criteria used in SRS scoring systems will be discussed in this chapter. IUGR was present in 76.7% (23/30) of pregnancies carrying the affected individual. Of the pregnancies in which IUGR was noted, the trimester of onset was reported in 22 cases. IUGR presented during the first trimester in 13.6% (3/22); the second trimester in 54.5% (12/22); and the third in 31.8% (7/22). The median gestational age at birth was 38 weeks (IQR 37-40) from medical records and 27.3% (9/32) were reported as being born 'early' from parental recollection. 78.8% (26/32) of individuals had a birth weight ≤-2 SDS. Relative macrocephaly at birth (head circumference SDS ≥1.5 above birth weight and/or birth length SDS) was present in 77.9% (7/9).

5.2.2 Neurodevelopment

The historical subjective concerns reported by parents about development included: concerns about early development in 56.7% (17/30); reaching motor milestones in 64.5% (20/31); and speech development in 38.7% (12/31). The median ages for objective developmental milestones from parental recall were as follows: sitting at 9 months (IQR 7-11), crawling at 10 months (7.36-12, n=7), bottom shuffling at 14.75 months (IQR 13-23, n=8), walking at 16 months (IQR 13-24, n=27) and talking at 12 months (IQR 10.25-21, n=16). Speech therapy had been received in 18.2% (6/33).

5.2.3 Educational support and attainment

The majority of individuals had attended mainstream education; mainstream nursery in 89.7% (26/29), mainstream primary school in 78.8 (26/33), and mainstream secondary school 72.7% (24/33). Special education had been accessed in some cases; nursery for children with special needs in 10.3% (3/29), mainstream primary school with special educational support in 21.2% (7/33), mainstream secondary with special educational support in 21.2% (7/33) and secondary school for children with special educational needs in 6.1% (2/33). These rates of special education needs are lower than the 14.4% prevalence of special educational needs reported in UK children (278). All three individuals who had attended a nursery for children with special needs, attended mainstream primary and secondary schools with special educational support in both settings. Four other individuals attended primary school with special educational support: two progressed to special needs secondary and two progressed to secondary school with special educational support. Of the two remaining individuals who attended secondary school with special educational support, one had not attended nursery but had attended mainstream primary; the other had attended both mainstream nursery and secondary.

27.3% (9/33) had a formal statement of educational need compared to 2.8% of children in the UK reported to have an educational statement (278). However, 45.5% (15/33) had received additional support at school. Educational/learning support was received by 6 individuals; support for nutrition (feeding) by 4 individuals; and physical support (related to short stature) by 11 individuals. In four cases, more than one form of support had been required; two forms in two cases and all three forms of support in two cases (Table 5.1).

Table 5.1 Additional help received at school by individuals with SRS.

ID number	Additional help at school	Additional educational help	Additional nutritional help	Additional physical help
1	no	no	no	no
2	no	no	no	no
3	yes	yes	no	yes
4	no	no	no	no
5	yes	no	no	yes
6	yes	no	no	yes
8	no	no	no	no
9	yes	no	no	yes
10	yes	no	yes	yes
11	yes	no	yes	no
13	no	no	no	no
14	no	no	no	no
16	yes	yes	yes	yes
17	yes	yes	no	no
18	yes	yes	no	no
19	no	no	no	no
22	no	no	no	no
23	no	no	no	no
24	no	no	no	no
25	no	no	no	no
26	no	no	no	no
27	no	no	no	no
29	no	no	no	no
31	yes	yes	no	no
32	yes	yes	yes	yes
33	no	no	no	no
34	yes	no	no	yes
35	yes	no	no	yes
36	no	no	no	no
37	yes	no	no	yes
38	no	no	no	no
40	yes	no	no	yes
41	no	no	no	no

5.2.4 Feeding and hypoglycaemia

The affected individuals were reported to have fed 'well as a newborn' in 15.6% (5/32) of cases. Conversely there was a history of 'poor appetite' in 84.4% (27/32). Nasogastric tube feeding was required in 59.4% (19/32) and gastrostomy feeding was reported in 9.1% (3/33).

Historical symptoms of excessive sweating and hypoglycaemia (recalled by parents) were reported in 61.3% (19/31) and 58.6% (17/29) of cases, respectively.

5.2.5 Movement disorders

There were no cases of movement disorders reported by individuals, parents or in the medical notes.

5.3 Clinical features of SRS in later life

5.3.1 Linear growth, BMI, asymmetry and relative macrocephaly

Height, weight and BMI of the cohort at final height are presented in Chapter 4. 60.6% (20/33) of the cohort had a height SDS ≤-2 SDS. Asymmetry, according to the definition used in the NHCSS, was present in 66.7% (22/33). Relative macrocephaly (current circumference SDS ≥ 1.5 above current height SDS) was present in 57.6% with a median difference between head circumference SDS and height SDS of 1.75 (IQR 0.45 to 2.98).

5.3.2 Dysmorphology

The following features were assessed at examination during the study appointment. Low set ears and posteriorly rotated ears were present in 57.6% (19/33) and 54.5% (28/33) respectively. Downslanting palpebral fissures were present in 30.3% (10/33). A broad nasal tip and broad nasal bridge were present in 21.2% (7/33) and 18.2% (6/33) respectively. Retro-/micrognathia was present in 31.8% (7/22). Facial asymmetry was present in 45.5% (15/33) and triangular facies were present in 25.8% (8/31). 2/3 toe syndactyly was present in 24.2% (8/33) and a wide sandal gap was present in 18.2% (6/33). Clinodactyly of the fifth toe or an overlapping fifth toe was present in 12.1% (4/33). Clinodactyly of the fifth finger was present in 78.8% (26/33). Joint hyperextensibility was reported in 19.4% (6/31).

The photographs of individuals with H19/IGF2 LOM are displayed in Figure 5.1. These photographs show that a broad forehead is commonly seen but the triangular facial shape described in infancy is less prevalent. In a few cases the gestalt of SRS remains evident whereas in the majority the facial features are less clear. Figure 5.2 displays the photographs of individuals with matUPD7. A broad forehead remains a facial feature and the three individuals in columns one and two also have triangular shaped facies.

Figure 5.1 Photographs of individuals with H19/IGF2 LOM. Columns 1 and 2: aged 13.3-15.4 years. Columns 3 and 4: aged 23.4-29.5 years. Columns 5-8 aged 31.2-40.3 years. Columns 9 and 10: aged 47.8-69.7 years. Permission for publication was granted for all images.

Figure 5.2 Photographs of individuals with matUPD7. Columns 1 and 2: aged 15.58-17.44. Columns 3 and 4: aged 22.03 to 33.94 years. Permission for publication was granted for all images.

5.3.3 Congenital anomalies

Congenital anomalies were present on examination or reported in medical notes in 55.5% (18/33) of cases. The number of congenital anomalies ranged from 0-5 with a median value of 1. Of the cases in which congenital anomalies were found, there was a single anomaly in 50.0% (9/18); two anomalies in 22.2% (4/18); three in 16.7% (3/18); four in one in 5.6% (1/18); and five in one in 5.6% (1/18).

Table 5.2 Congenital anomalies in the UK STAARS cohort. Data on congenital anomalies, cleft palate/bifid uvula, female genital anomalies and male genital anomalies presented as percentage and raw data. Data on number of congenital anomalies presented as median (range) with number (n) shown. All other data presented as number detected.

	All molecularly confirmed	H19/IGF2 LOM	matUPD7	P value
Congenital anomalies	54.5 (18/33)	63.0 (17/27)	16.7 (1/6)	0.070
Number of congenital anomalies (median,range)	1.5 (1-5) (n=18)	1 (1-5) (n=17)	3	0.278
Cleft palate/bifid uvula	9.1 (3/33)	11.5 (3/27)	0	0.556
Female genital anomalies	16.7 (3/18)	20.0 (3/15)	0/3	1.000
Male genital anomalies	33.3 (5/15)	41.7 (5/12)	0/3	0.505
Cardiac anomalies	3	2	1	1.000
Renal anomalies	3	2	1	1.000
Radial anomalies	1	1	0	1.000
Thumb anomalies	2	2	0	1.000
Coloboma	1	1	0	1.000
Kyphoscoliosis	8	7	1	0.663
Limited elbow supination/congenital dislocation	3	3	0	0.614
Camptodactyly	5	5	0	0.556
Brain anomaly	1	1	0	1.000

Table 5.2 shows the specific major, congenital anomalies and numbers detected. These categories of anomalies have been described in previous SRS cohorts (21, 80). Of the three individuals with cleft palate, a bifid uvula was present in one of these cases. The genital anomalies in females included: 1) vaginal agenesis with a hypoplastic uterus and single ovary; 2) hypoplastic genitalia with pronounced labia minora and a history of vaginal hernia; and 3) a bicornuate uterus with double cervix. The genital anomalies in males included: 1) a history of bilateral cryptorchidism in four cases and 2) a history of ambiguous genitalia and severe hypospadias. The cardiac anomalies were comprised of: 1) tricuspid valve regurgitation; 2) cardiac juxtaposition; and 3) history of coarctation of the aorta with multiple ventriculo-septal defects. The renal anomalies reported were: 1) a solitary kidney with crossed fused ectopia 2) horseshoe kidney, and 3) malrotation of

one kidney. Radial anomalies were present in three cases; hypoplasia of the radii with absent thumbs bilaterally, a case with a bifid thumb, which had been surgically corrected and one case of congenital dislocation of the radial head. In addition, there were two cases of limited elbow supination. Scoliosis was present in 24.2% (8/33) individuals and in one case was associated with kyphosis. There was one case of 'congenital hip dislocation' which is now termed developmental dysplasia of the hip. Camptodactyly affecting distal interphalangeal joints was present in five cases. A coloboma of the iris was present in one individual. The brain abnormality detected in one individual was reported as a mildly dysplastic corpus callosum.

5.3.4 Feeding and hypoglycaemia

Reports of feeding patterns in later life included two individuals who stated that they perceived hunger to a greater degree as adults than they had as children, two cases where hunger was constantly experienced, four cases in which individuals described themselves as fussy or difficult with food, and seven cases in which the appetite was described a 'good' or 'large' or there was an allusion to eating large or excessive volumes.

One individual continued to experience the symptoms of hypoglycaemia, including unresponsive episodes, despite biochemical normoglycaemia and extensive investigations had not revealed a cause.

5.3.5 Medical conditions

Of the 33 individuals with H19/IGF2 LOM and matUPD7, there was a broad range of diagnosed medical conditions and symptoms (as reported by individuals during the research appointment, reported by parents at the appointment or via questionnaire and obtained from medical notes).

Table 5.3 shows the number of individuals (and percentage prevalence) affected by various numbers of medical conditions.

Table 5.3 Overview of medical conditions in the STAARS UK cohort.

Number of medical conditions	Numbers of individuals	Percentage of STAARS UK cohort
0	2	6.1
1	1	3.0
2	4	12.1
3	3	9.1
4	2	6.1
5	7	21.2
6	4	12.1
7	1	3.0
8	1	3.0
9	2	6.1
10	1	3.0
11	2	6.1
12	1	3.0
13	1	3.0
14	1	3.0
Total	33	100

Table 5.4 Number of medical conditions diagnosed for each individual stratified by physiological system or symptom group.

ID number	Endocrinology /metabolism	Ear nose and throat	Gynaecology	Psychiatry	Respiratory	Musculoskeletal	Surgery	Fractures	Arthralgia/ myalgia	Dental treatment	Infections	Atopic conditions	Dermatology	Neurology	Haematology	Gastrointestinal	Renal	Ophthalmology	Collapses	Cardiovascular	Food allergy/intolerance	Audiology
1							1				1											
2			1	1			1		1	1		1	1	2					1	1		
3			1		1	3	3						1					1		1		
4		1	1			1									1							
5	2			1	1	2			1	1						3				1		
6					1					1	1											
8																						
9		1		2						1								1				
10					1			2		1												
11	1					1	1				2								1			
13		1						1	1		1		2						1		3	
14				1					1	1												
16					1	1		1		1											1	
17					1																	1
18				2			1			1				3		2						
19					1	1	2	1														
22							1		1	1	1			1		2						
23	1													1				1				
24									1	1												
25	2					2				1							1					
26					1		1	1		1			1								1	
27			2	3				3	2		1			1							2	
29	1				2	3						1	1	1		1				1	1	1
31																						
32		2				1	3		1	1	1											
33							2	1				1		1								
34									1	1	2				1							
35										1	1											
36							2	1						2		1						
37	1				1	1										2						
38					1	2	1	1	1					1					1			
40					1	2				1			1									
41																				1		

Table 5.4 shows the number of medical conditions of each system or symptom diagnosed for each participant. All conditions or operations detailed below are included in the above table.

There was a history of respiratory disorders in 36.4% (12/33). These included asthma (n=10), restrictive lung disease (n=1), bronchiectasis (n=1), primary pulmonary hypertension (n=1). In one case, there were two co-existing conditions.

The following cardiovascular signs or conditions were reported (each affecting one individual): 1) postural/orthostatic hypotension, which required treatment with fludrocortisone and midodrine; 2) pulmonary valve regurgitation 3) unspecified valvular regurgitation (historical medical notes unavailable), 4) hypertension and 5) a heart murmur although previously had a normal echocardiogram therefore may have been a flow murmur.

The following infections were reported: recurrent infections (n=2); frequent chest infections (n=2); recurrent bronchiolitis (n=1); meningitis (n=3); frequent ear infections (n=3). One individual who had recurrent infections also had viral meningitis.

Ear, nose and throat problems were diagnosed in 12.1% (4/33) of cases. Frequent or recurrent ear infections were reported in three cases, a history of grommet insertion in a further two cases and a history of glue ear in one case. One individual who had frequent ear infections had also been diagnosed with bilateral ear canal stenosis and recurrent tonsil stones.

Endocrine/metabolic problems were present in 18.2% (6/33). Hypothyroidism was diagnosed in two cases (6.1%). Biochemically confirmed hypoglycaemia was reported in two cases, with one being recurrent. Two individuals had been diagnosed with type 2 diabetes mellitus, one with impaired glucose tolerance, and one with hypercholesterolaemia.

Mental health problems were reported in 18.2% (6/33). Diagnoses included depression (n=4), anorexia and bulimia (n=1), attention-deficit hyperactivity disorder (n=1) and panic attacks (n=2). In three cases, there were co-existing conditions.

A history of musculoskeletal problems was present in 36.4% (12/33). Problems were comprised of: hypermobility (n=2), trigger finger (n=1), Raynaud's syndrome (n=2), a ganglion cyst (n=1), childhood rheumatoid arthritis (n=1), anterior cruciate ligament tear (n=1), locking knees (n=1), joint dislocations (n=3), pes cavus (n=2), osteopenia (n=2), osteoarthritis (n=1), patella alto (n=1) and prolapsed vertebral disc (n=1). In six cases, there were co-existing conditions.

In addition to surgery to correct the congenital anomalies discussed in section 5.3.3, there were additional surgical diagnoses in 36.4% (12/33). These included herniae (n=3) (two specified as

inguinal), leg lengthening (n=2) and subsequent femoral epiphysiodesis in one of these cases, ligament and/or tendon surgery (n=2), pinnaplasty (n=3), gastrostomy and reversal (n=2), rhinoplasty (n=1), sterilisation (n=1) and jaw surgery (n=1). One individual had a history of malignant hyperthermia at induction of anaesthesia.

Arthralgia/myalgia were reported as 'joint pains' or 'aches', which affected the back, hip, neck, knees and fingers where specified, were reported in 30.3% (n=10) individuals. The severity of pain affected mobility in two of these cases, who each used a wheelchair for travelling long distances. One of the two wheelchair users described 'an myalgic-encephalomyelitis-like illness' in the past, and problems subsequently. Another individual (of the ten reporting joint pains or aches) had been diagnosed with chronic fatigue syndrome.

Dental treatment was reported in 48.5% (16/33) with braces having been required in seven cases, tooth extraction in 12 cases, dental operations reported in one case and unspecified treatment in one case.

There was a history of food allergy or intolerance in 15.2 % (5/33). Two of these individuals had peanut allergy; one an idiopathic food allergy; and two had dairy and soya intolerance with one of these individuals also having an intolerance to egg. Three individuals reported a history of hayfever. There was a history of skin conditions in 18.2% (6/33) with eczema in three individuals, acne in two individuals, psoriasis in one and a history of both eczema and acne in other case.

A history of neurological problems was present in 27.3% (9/33). This included migraines (n=3), headaches (n=2), sciatica (n=2), Bell's palsy (n=1), ptosis (n=1), cerebral atrophy and seizures (n=1), recurrent bilateral optic neuritis in one case who also had a transient ischaemic attack.

There were histories of dizziness, faints or recurrent collapses in four individuals (12.1%), one of whom also had postural hypotension. Hospital notes were available for two of the remaining three individuals: one had been investigated for long QT syndrome and the other for recurrent unresponsive episodes, with biochemical normoglycaemia but responding to treatment with glucose.

Gastro-intestinal disorders had been diagnosed in 18.2% (6/33). There was a history of gastro-oesophageal reflux in five individuals, one of whom had been demonstrated to have delayed gastric emptying. Irritable bowel syndrome was diagnosed in one case and a possible diagnosis in another. In one individual, biliary reflux and non-alcoholic hepatitis were reported (the latter will be discussed further in Chapter 7).

Ophthalmological problems were reported in 9.1% (3/33); two squints and one case of bilateral astigmatism. Two individuals (6.1%) had been diagnosed with hearing loss. In one case this was unilateral and in the other case hearing loss was bilateral.

There were two haematological diagnoses reported (6.1%), which were Factor V Leiden deficiency and anaemia (of unknown aetiology). In additional to renal anomalies discussed in section 5.3.3, one further renal disorder was reported; proteinuria in one individual (3.0%).

Movement disorders were not reported and no tremors were identified on examination.

5.3.6 Haematological and biochemical investigations

Following venepuncture during the research study visit, blood samples were sent for investigations including: full blood count, renal profile, liver function tests, and thyroid function tests and insulin-like growth factor 1 (IGF1).

Table 5.5 Results from haematological and biochemical investigations on STAARS UK cohort. IQR; interquartile range.

	Median (IQR)
Haemoglobin (g/L)	147.5 (134.3-156.8)
White blood count (10*9/L)	6.65 (5.60-9.35)
Platelets (10*9/L)	257 (229.3-294.5)
Sodium (mmol/L)	139.0 (137.0-140.0)
Potassium (mmol/L)	4.20 (4.00-4.00)
Urea (mmol/L)	5.10 (4.45-5.85)
Creatinine (µmol/L)	56.0 (47.0-71.5)
Total protein (g/L)	74.0 (71.8-77.5)
Albumin (g/L)	43.0 (41.0-46.0)
Bilirubin (µmol/L)	10.0 (6.0-13.5)
Alkaline phosphatase (U/L)	88.0 (72.5-121.5)
TSH (mU/L)	1.83 (1.27-2.37)
Free T4 (pmol/L)	11.7 (10.4-13.0)

Table 5.5 shows the results overall. The following results are reported according to the reference ranges provided by the Pathology Department at University Hospital Southampton. There were two cases of raised haemoglobin (2/32). There were two cases of raised white blood cell count (2/32). These results were obtained within the context of viral illnesses in the participants. Platelet counts were within normal limits in all cases (n=32). There was one case of hyponatraemia with a sodium level of 128 mmol/L with a normal potassium level of 4.40 mmol/L

and normal renal function. This individual was taking medications including amlodipine, doxazosin, fexofenadine, carbocysteine, omeprazole, glycopyrronium, montelukast, meloxicam and metformin. The hyponatraemia was known to the individual and no cause had been found despite investigation. Potassium levels were within the reference ranges (n=31). In one case, urea was raised to 7.80 mmol/L with a normal creatinine level (1/33). Creatinine levels were low in 54.5% (18/33) and are discussed further in section 7.4. Thyroid stimulating hormone (TSH) levels were normal (n=31) and free T4 levels were within the laboratory reference ranges (n=31) including the cases diagnosed with hypothyroidism, which would reflect appropriate treatment.

The following investigations will be presented and discussed in section 7.4 as part of the cardio-metabolic assessment: fasting glucose, glycosylated haemoglobin, insulin, C-peptide, alanine aminotransferase, aspartate aminotransferase, gamma glutamyl transferase, cholesterol and triglycerides.

5.3.7 Genitourinary anomalies, fertility, offspring and SRS risk

72.7% (24/33) participants had no offspring. Nine individuals (5 females; 4 males) had children: three had one offspring; four had two; and two had three. The molecular diagnoses of the individuals with offspring were: H19/IGF2 LOM in eight cases and matUPD7 in one case. In no case was the child of a proband affected with SRS.

In addition to the three females with genital anomalies described in section 5.3.3, there was a history of gynaecological problems in 22.2% (4/18) of females, including endometriosis in one case, menorrhagia in two cases and pelvic inflammatory disease likely secondary to chlamydia in one case. In women of reproductive age, endometriosis has been reported in 10-15% (279) and menorrhagia in 30% (280).

One female reported infertility in that the duration of time to conceive her first child was three years, however no treatment had been received. This compares to a reported prevalence of infertility (defined as unsuccessfully attempting pregnancy for one year or longer) of 12.5% in women (281). Miscarriages had been experienced by two females (one and two miscarriages in each case).

Primary hypogonadism, azospermia and infertility had been confirmed in one male, who had a history of ambiguous genitalia including severe hypospadias. One of the four males with a history of cryptorchidism and orchidopexy, was noted to have small testicular volumes during puberty, a borderline testosterone level and a raised follicle stimulating hormone level (FSH). No results from semen analysis were available. One male with a history of cryptorchidism was father to two

children. One further male, who had been diagnosed with testosterone deficiency with low testosterone levels but normal FSH and lutenising hormone, had one offspring. This individual also reported prostate problems. The prevalence of infertility (defined as unsuccessfully attempting pregnancy for one year or longer) reported in men has been reported as 10.1% (281). The prevalence of cryptorchidism in the general population has been reported as 1.6-2.2% of boys aged ≥15 years and a shortage of studies performed at an older age has been acknowledged (282). The global prevalence of testosterone deficiency has been estimated at 10-40% (283).

5.3.8 Educational attainment in SRS

General Certificates of Secondary Education (GCSEs) or equivalents, including Certificates of Secondary Education (CSEs) and General Certificates of Education Ordinary level (O-levels), were attained in 92.6% (25/27) of eligible cases (i.e. aged ≥16 years). General Certificates of Education Advanced levels (A-levels) or equivalents, including Business and Technology Education Council (BTEC) qualifications, were gained in 56.0% (14/25) of eligible cases (i.e. aged ≥18 years). University degrees were completed in 40.0% (10/25) of eligible cases (i.e. aged ≥21 years) and one degree-level BTEC was achieved. This compares to 42% of the UK population aged 21-64 with higher educational qualifications (284).

There was no association between historical reported concerns about early development and GCSE attainment (p=0.326) or degree attainment (p=0.181), however A-level attainment was less likely in cases where there had been concern about early development than where there had been no concern (11.8% vs 76.9%, p<0.001). There was no association between historical reported concern about reaching normal motor milestones and GCSE attainment (p=0.516) or degree attainment (p=0.221), however A-level attainment was less likely in cases where there had been concern regarding reaching milestones (25.0% vs 72.7%, p=0.014). There were no associations between historical reported concerns about speech development and GCSE attainment (p=0.241), A-level attainment (p=0.347), or degree attainment (p=0.611).

5.4 Epigenotype-phenotype correlations

There was a suggestion that the median maternal age of individuals with matUPD7 (33.72 years, IQR 28.10-36.06) was higher than the median maternal age of individuals with H19/IGF2 LOM (29.44 years, IQR 26.07-31.68) (p=0.148). There was also a suggestion that gestational ages at birth were lower in matUPD7 cases (median 37.5 weeks, IQR 35.0-38.3) compared to H19/IGF2 LOM cases (median 39.0 weeks, IQR 37.0-41.0) (p=0.130). IUGR occurred more frequently in H19/IGF2 LOM than matUPD7; 87.5% (n=24) and 33.3% (n=6) respectively (p=0.016). There was

no difference between H19/IGF2 LOM and matUPD7 in the trimester in which IUGR was detected (p=0.216). Birth weight was lower in H19/IGF2 LOM cases compared to matUPD7 cases; median -3.54 (IQR -4.20 to -2.64) and -2.19 (IQR -2.98 to -1.29), p=0.029. This corresponded with a greater proportion of H19/IGF2 LOM cases than matUPD7 cases having a birth weight SDS ≤-2 SDS; 88.5% vs 50% (p=0.063). There was no significant difference in adult relative macrocephaly between the epigenotype groups. Asymmetry was observed more commonly in H19/IGF2 LOM than in matUPD7; 77.8% vs 16.7% (p=0.01).

There were no significant differences between H19/IGF2 LOM and matUPD7 cases in reported concerns about speech development, rates of speech and language therapy, or concerns about early development. There were reported concerns about reaching normal motor milestones in all matUPD7 cases (6/6) but only 56.5% (14/25) H19/IGF2 LOM cases (p=0.066). One third of the individuals who attended special needs nursery had matUPD7. Educational support at school was received by 50% (3/6) of individuals with matUPD7 and 11.1% (3/27) individuals with H19/IGF2 LOM (p=0.058).

A higher proportion of matUPD7 cases reported a history of hypoglycaemia than H19/IGF2 LOM cases; 100% (6/6) vs 47.8% (11/23), (p=0.056). Similarly, a higher proportion of matUPD7 cases reported a history of excessive sweating than H19/IGF2 LOM cases; 100% (6/6) vs 52.0% (13.25) (p=0.059).

Apart from two congenital anomalies (in one individual with matUPD7), all other congenital anomalies (n=35) were found in individuals with H19/IGF2 LOM. This meant that congenital anomalies were present in 63.0% (17/27) individuals with H19/IGF2 LOM and 16.7% (one individual) of the matUPD7 group (p=0.07). There were no distinguishing findings on assessment of facial features and foot morphology. However, clinodactyly was present in 88.9% (24/27) of H19/IGF2 LOM cases versus 33.3% (2/6) matUPD7 cases (p=0.011).

The clinical photographs in Figure 5.1 and Figure 5.2 show that downturned corners of the mouth, downturned palpebral fissures and asymmetry can be seen in some individuals with H19/IGF2 LOM and are less frequently seen in individuals with matUPD7. However there appear to be an increased prevalence of triangular facies in matUPD7 compared to H19/IGF2 LOM.

5.5 Sex-phenotype associations

As discussed in section 3.1, there was a qualitative arm of the STAARS study. Individuals discussed their lived experience of SRS and a theme – of joint pains and aches – emerged in females. Quantitative analysis was undertaken to examine the joint pains and aches reported within the

medical histories (see section 5.3.5). 44.4% (8/18) of females reported joint pains and aches compared to 6.66% (1/15) of males (p=0.021). The estimated prevalence of musculoskeletal conditions in females and males respectively is 25.9% and 31.8% (285). However, that estimate included health problems affecting the bones, joints, muscles and spine.

5.6 Application of SRS scoring systems to the STAARS UK cohort

5.6.1 SRS scoring systems in the STAARS UK cohort

The proposed SRS scoring systems were discussed in section 1.11. Each system was applied to the genetically confirmed SRS cases in this cohort. This was first undertaken using data available at examination and then second, undertaken using additional historical data as described in section 3.10.

The scoring system proposed by Lai et al (23) diagnosed 54.5% (18/33) cases with immediately available data and 72.7% (24/33) with additional historical data. The scoring system proposed by Price et al (20) diagnosed 15.2% (5/33) cases with immediately available data and 45.5% (15/33) with additional historical data. The scoring system proposed by Netchine (24) diagnosed 54.5% (18/33) cases with immediately available data and 72.7% (24/33) with additional historical data. The scoring system proposed by Bartholdi (34) diagnosed 54.5% (18/33) cases with immediately available data and the same number with additional historical data. The scoring system proposed by Dias (35) diagnosed 51.5% (17/33) cases with immediately available data and 63.6% (21/33) with additional historical data. The NHCSS proposed by Azzi et al (32) diagnosed 18.2% (6/33) cases and a further 27.3% (9/33) cases as 'possible SRS' with immediately available data. Five out of the six criteria from which it is determined were available in 90.9% (30/33) cases with the remaining cases having four criteria available. With the inclusion of historical data, SRS was diagnosed in 39.4% (13/33) and a further 33.3% (11/33) cases as 'possible SRS'. All six criteria were available in 87.9% (29/33) of cases and five out of the six criteria were available in the remaining 12.1% (4/33) cases.

5.6.2 Focus on SRS scoring systems in adults in the STAARS UK cohort

In those aged ≥18 years (n=25), the scoring system proposed by Lai et al (23) diagnosed 56.0% (14/25) cases with immediately available data and 76.0% (19/25) with additional historical data. The scoring system proposed by Price et al (20) diagnosed 12.0% (3/25) cases with immediately available data and 48.0% (12/25) with additional historical data. The scoring system proposed by Netchine (24) diagnosed 60.0% (15/25) cases with immediately available data and 76.0% (19/25)

with additional historical data. The scoring system proposed by Bartholdi (34) diagnosed 52.0% (13/25) cases with immediately available data and the same number with additional historical data. The scoring system proposed by Dias (35) diagnosed 60.0% (15/25) cases with immediately available data and 72.0% (18/25) with additional historical data. The NHCSS proposed by Azzi et al (32) diagnosed 16.0% (4/25) cases and a further 32.0% (8/25) cases as 'possible SRS' with immediately available data. Five out of the six criteria were available in 92.0% (23/25) cases with the remaining cases having four criteria available. With the inclusion of historical data, SRS was diagnosed in 40.0% (10/25) and a further 40.0% (10/25) cases as 'possible SRS'. All six criteria were available in 88.0% (22/25) cases and five out of the six criteria were available in the remaining three cases.

5.6.3 Focus on SRS scoring systems in adolescents in the STAARS UK cohort

For those aged <18 years (n=8), the scoring system proposed by Lai et al (23) diagnosed 50.0% (4/8) cases with immediately available data and 62.5% (5/8) with additional historical data. The scoring system proposed by Price et al (20) diagnosed 25.0% (2/8) cases with immediately available data and 37.5% (3/8) with additional historical data. The scoring system proposed by Netchine (24) diagnosed 37.5% (3/8) cases with immediately available data and 62.5% (5/8) with additional historical data. The scoring system proposed by Bartholdi (34) diagnosed 62.5% (5/8) cases with immediately available data and the same proportion with additional historical data. The scoring system proposed by Dias (35) diagnosed 25.0% (2/8) cases with immediately available data and 37.5% (3/8) with additional historical data. The NHCSS proposed by Azzi et al (32) diagnosed 25.0% (2/8) cases and a further 12.5% (1/8) cases as 'possible SRS' with immediately available data. Five out of the six criteria were available in 87.5% (7/8) cases with the remaining case having four criteria available. With the inclusion of historical data, SRS was diagnosed in 37.5% (3/8) and a further 12.5% (1/8) cases as 'possible SRS'. All six criteria were available in 87.5% (7/8) cases and five out of the six criteria were available in the remaining case.

5.7 Clinical SRS cases

5.7.1 Application of NHCSS in clinical SRS cases

There were four individuals with clinical diagnoses of SRS (testing negative). Application of the NHCSS to their clinical features is shown in Table 5.6. One individual meets the criteria for diagnosis of SRS (32). Two individuals meet the criteria for 'possible SRS' (15). Clinical photographs of these four individuals are shown in Figure 5.3.

Figure 5.3 Photos of clinical SRS cases aged 19.69 to 28.86 years. Permission for publication was granted for all images.

Table 5.6 The Netchine-Harbison clinical scoring system applied to clinical SRS cases. SGA defined as birth weight and/or birth length ≤-2SDS). Relative macrocephaly defined as OFC SDS ≥1.5 above height SDS. †forehead obscured on photograph.

	Case ID 7	Case ID 12	Case ID 15	Case ID 39
SGA	Yes	Yes	Yes	No
Height ≤-2 SDS	Yes	No	Yes	Yes
Relative macrocephaly on examination	No	No	Yes	Yes
Protruding forehead on examination	No	Unable to assess from photograph†	No	No
Body asymmetry at time of examination	No	Yes	Yes	Yes
History of feeding difficulties and/or BMI ≤-2 SDS	Yes	No	No	No
NHCSS total	3/6	2/5	4/6	3/6
SRS diagnosis	Possible SRS	SRS unlikely	SRS diagnosed	Possible SRS

5.8 Cases of maternal uniparental disomy for chromosome 14

5.8.1 Comparison of phenotype

As discussed in section 1.12.1, a molecular genetic diagnosis of matUPD14 is associated with Temple syndrome, which has clinical features in common with SRS. As planned in section 4.5.3, matUPD14 cases are presented and discussed separately in this section.

Table 5.7 depicts the clinical features of the three individuals with matUPD14 reviewed in this study and comparison to individuals with H19/IGF2 LOM in the STAARS UK cohort. Individuals with matUPD14 had higher birth weights, reduced prevalence of asymmetry (none) and increased special educational needs compared to those with H19/IGF2 LOM. Clinical photographs of the individuals with matUPD14 are shown in Figure 5.4. A broad forehead and almond-shaped eyes can be seen.

Table 5.7 Clinical characteristics of individuals with H19/IGF2 LOM and matUPD14. P values in bold denote significance.

	H19/IGF2 LOM	matUPD14	P value
Number	27	3	
Sex			
Males	44.4 (12)	33.3 (1)	
Female	55.6 (15)	66.6 (2)	1.000
Age (median, range)	32.35 (13.36-69.71)	24.65 (13.53-28.52)	
BW SDS (median, range)	-3.54 (-7.76 to -1.52) (n=26)	-2.41 (-2.59 to -1.39)	
BW SDS (median, IQR)	-3.54 (-4.20 to -2.64) (n=26)		**0.022**
BW SDS ≤-2 (%)	88.5 (23/26)	66.7 (2/3)	0.371
Relative macrocephaly, 1.5 (%)		50.0 (1/2)	0.378
Relative macrocephaly, 1.0 (%)		50.0 (1/2)	
Height SDS (median, range)		-3.79 (-4.28 to -2.21)	
Height SDS (median, IQR)	-3.13 (-3.87 to -1.02)		0.467
Height SDS ≤-2 (%)	63.0 (17/27)	100 (3/3)	
Weight SDS (median, range)		-0.85 (-1.36 to 2.14)	
Weight SDS (median, IQR)	-1.83 (-4.66 to -0.11)		0.315
BMI SDS	-0.80 (-1.99 to 1.49)	1.33 (0.40 to 3.53)*	0.086
Obesity	11.5 (3/26)	33.3 (1/3)	0.371
Adult relative macrocephaly, 1.5 (%)	55.6 (15/27)	66.7 (/3)	1.000
OFC SDS (median, IQR)	-0.97 (-1.85 to -0.44)	-1.25 (-1.55 to -0.52)	1.000
NHCSS asymmetry (%)	77.8 (21/27)	0	**0.021**
Statement of educational needs (%)	25.9 (7/27)	66.7 (2/3)	0.207
Attended special needs nursery	8.7 (2/23)	33.3 (1/3)	
Attended mainstream nursery	91.3 (21/23)	66.7 (2/3)	0.319
Attended mainstream primary with special help	18.5 (5/27)	33.3 (1/3)	
Attended mainstream primary	81.5 (22/27)	33.3 (1/3)	**0.043**
Attended special needs secondary	7.4 (2/27)	33.3 (1/3)	
Attended mainstream secondary with special help	14.8 (4/27)	0	
Attended mainstream secondary	77.8 (21/27)	66.7 (2/3)	0.315
Attained GCSEs or equiv (aged 16 and over)	90.9 (20/22)	50.0 (1/2)	0.207
Attained A-levels or equiv (aged 18 and over)	54.5 (12/22)	50.0 (1/2)	1.000
Attained Degree (aged 21 and over)	45.5 (10/22)	0 (0/2)	0.532

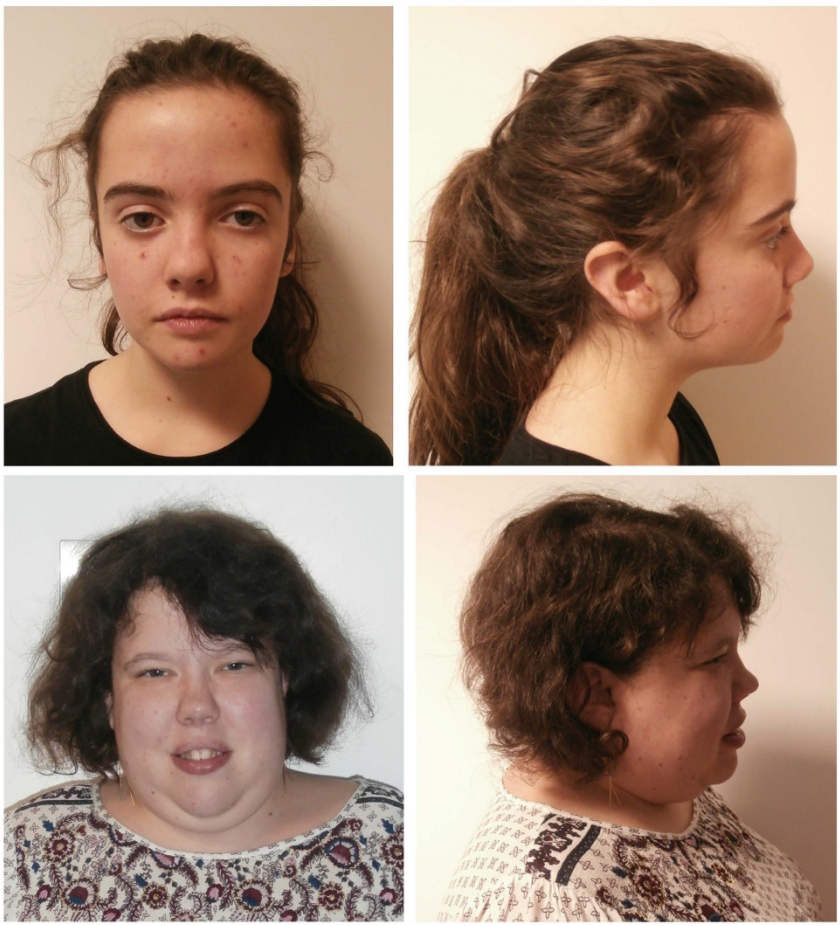


Figure 5.4 Photographs of individuals with matUPD14 aged 13.53 and 28.52 years. Permission for publication was granted for all images.

5.8.2 Application of NHCSS in maternal uniparental disomy for chromosome 14 cases

Application of the NHCSS to their clinical features is shown in Table 5.8. In two cases the criteria for 'possible SRS' are met and in the other case, the score indicates SRS to be unlikely. These results are discussed in section 5.9.6.

Table 5.8 The Netchine-Harbison clinical scoring system applied to cases of matUPD14. SGA defined as birth weight and/or birth length ≤-2SDS). Relative macrocephaly defined as OFC SDS ≥1.5 above height SDS.

	Case ID 20	Case ID 21	Case ID 30
SGA	Yes	Yes	No
Height ≤-2 SDS	Yes	Yes	Yes
Relative macrocephaly on examination	Yes	Yes	No
Protruding forehead on examination	No	No	No
Body asymmetry at time of examination	No	No	No
History of feeding difficulties and/or BMI ≤-2 SDS	No	No	No
NHCSS total	3/6	3/6	1/6
SRS diagnosis	Possible SRS	Possible SRS	SRS unlikely

5.9 Discussion

This study represents one of the largest deep phenotyping studies of adults with SRS. The results are based in part on very detailed examination but also historical medical notes and discussion with family members. All the results from the early life period are based on participant or parent recall and hospital notes. As discussed in section 4.4, the early growth pattern of the STAARS UK cohort is comparable to previously reported SRS cohorts that have concentrated on the childhood aspect of this disorder.

5.9.1 Clinical features of SRS in early Life

At the time of birth of the study participants, the median maternal age was 30.52 years and the median paternal age was 32.49 years. Proportion of parents of cases affected by infertility or pregnancy loss was 38.7%. Overall rate of assisted reproductive technology in proband pregnancy was 6.45%. The 76.7% prevalence of IUGR in this cohort lies between the 58% reported by Price et al (20) and 89% reported by Wakeling et al (21). In these reports, IUGR was noted before 26 weeks in 11 of 29 cases (20) and at an average gestational age of 23 weeks (21). The majority of cases of IUGR in this study were detected in the second trimester, which is likely to reflect the timing of antenatal ultrasound scanning, with a first scan usually performed at the end of the first trimester and subsequent scanning at approximately 20 weeks. The 22.6% prevalence of preterm births in this cohort is higher than the rate of 16% (8/50) births between 31 and 36 weeks reported by Price et al (20) and the rate of 7.3% estimated in the UK in 2012 (286). However, the preterm births described here also resulted from elective and emergency Caesarean sections. The indications for Caesarean sections were unclear but there may have been concern about IUGR and decisions made to intervene to prevent pregnancy loss in the case of placental insufficiency. The median gestational age at birth of 38 weeks is very close to the mean gestational age at birth previously reported 37.8 ± 3.2 weeks (46). The 78.8% with birth weight ≤-2 SDS and 77.9% prevalence of relative macrocephaly at birth are almost identical to the 78% reported by Wakeling et al (21).

Overall this cohort therefore conforms to an established pattern with regard to birth history, supports the methods used to ascertain these data, and suggests that the results regarding the adult phenotype are applicable to wider cohorts of individuals with SRS.

5.9.2 Fertility in SRS

For the first time this cohort has confirmed that pregnancy for people with SRS is possible and the finding that four males and five females have had their own children is useful for families growing

up with this condition. The fact that none have had children with SRS is in keeping with the literature that there is a very low recurrence risk for people with SRS (15).

In this cohort, infertility in the pregnancy leading to the birth of the proband was reported in 18.8% which is the same as the overall rate of 18.8% (taking >1 year to conceive) in the study by Wakeling et al (21). The 6.45% rate of ART in this cohort is above the general UK rate of 1.8-2.4% (287, 288). An increased frequency of SRS has been reported after ART (289). In two cohorts of SRS cases, 6.6-11% were conceived by ART (21, 35). In one study 1/7 ART-conceived patients had H19/IGF2 LOM (35) whereas in the other, all SRS cases born after ART had H19/IGF2 LOM (21). Similarly, in the STAARS UK cohort, both cases born by IVF and IVF ICSI had H19/IGF2 LOM. An association between IVF and LOM is predicted because epigenetic marks, such as methylation, are required to withstand the early cell divisions of the zygote (as described in sections 1.10.2 and 1.10.3). When these early cell divisions occur in vitro epigenetic aberrations may result (290).

5.9.3 Development and educational attainment in SRS

The highest proportion of concern regarding development related to motor development. The prevalence of global developmental delay has been reported as 34% of a cohort and motor delay reported as common with a mean age at walking of 20 months (21). Participants in this cohort also demonstrated a tendency to later walking with the median age at walking being 16 months.

The proportion of individuals in this study (27.3%) who had a statement of special educational need was within the range of 15-38% previously reported (20, 21, 23). This is greater than the 2.8% of children in the UK reported to have a statement of special educational need (278). The proportion of individuals in this cohort who had attended a school for children with special educational needs (10.3% for nursery and 6.1% for secondary school) was comparable to the 8-10.5% of children with SRS previously reported (20, 23) and below the 14.4% of children reported to have special educational needs in the UK (278). A similar proportion of individuals had received additional help within a mainstream school: 21.2% in primary school and 21.2% in secondary school compared to 28% in a previous study (23). Help with reading and language had previously been reported (23), however, in this study additional support for nutritional needs and physical support were also described. Speech therapy in this cohort had been received in a lower proportion of cases than in previous studies; 18.2% compared to 26-48% (20, 23). Although speech therapy has been demonstrated more commonly in individuals with matUPD7 than those with H19/IGF2 LOM (21), no significant difference was demonstrated in this study. This may be as a result of the small number of individuals with matUPD7 included in this cohort. Educational attainment/qualifications have not previously been reported. Interestingly there was no

association between historical reported concern about attainment of normal motor milestones and GCSE attainment although A-level attainment was less likely where there had been concerns. This may reflect that severe motor delay might be associated with a degree of learning impairment but that the learning impairment is mild. There was a markedly high entry to University (40%) which is comparable to the 42% of the UK population aged 21 to 64 who had achieved higher education qualifications in 2017 (284).

5.9.4 Congenital anomalies in SRS

Congenital anomalies have been reported more commonly in cases of H19/IGF2 LOM compared to matUPD7 (21, 80). In this cohort, apart from one individual with matUPD7, congenital anomalies were present or there was a positive history in individuals with H19/IGF2 LOM. Although the difference appeared striking, it did not reach statistical significance, which may be as a result of the small number in the matUPD7 group. Camptodactyly has been suggest to advance in severity with increasing age (21) but this was not demonstrated in this cohort. Limitation to upper limb extension has been reported individuals with H19/IGF2 LOM and was similarly found in this cohort (21).

5.9.5 Clinical features in later life in SRS

In this cohort, there was a history of poor appetite in 84.4%, nasogastric tube feeding in 59.4% and gastrostomy tube feeding in 9.1% but feeding problems at the time of the research appointment tended to relate to excessive intake. These findings mirror the observation that feeding problems and the requirement for enteral feeding in SRS appears to reduce with increasing age (27). A PubMed search using the terms ‘age-related’ and ‘changes’ and ‘appetite’ produced publications related to anorexia in the elderly rather than changes in appetite from childhood to adulthood. A gradual increase in body weight through adulthood until approximately age 60-65 years has been reported with a subsequent decline after age 65-75 years with the decline in body weight believed to be related to ‘anorexia of ageing’ (291).

The prevalence of historical symptoms of excessive sweating described in 61.3% and perceived hypoglycaemia in 58.6% are comparable to reports of excessive sweating reported by parents in 67% of a previous study (Wakeling) (21) and these had mostly not been formally investigated for hypoglycaemia.

A previous study reported movement disorders in 10% (2/20) individuals with matUPD7. In one case, there was a history of myoclonic jerks between three weeks and one year of age and the other individual aged 14.9 years experienced intermittent head shaking episodes (21). No cases

were found in this cohort, which may again have been affected by the limitation in the number of individuals with matUPD7 recruited.

The facial features of individuals with SRS have been acknowledged to become less evident with increasing age (21, 32). The facial features in older ages differ from young children affected with SRS; a broad forehead remains typical, however triangular facies are less evident and appear to be more common in matUPD7 cases.

Joint problems, such as camptodactyly, contractures and limited elbow supination, have previously been described in SRS. However, arthralgia and muscle pain have neither been described in the frequency seen in this cohort nor attributed to females with SRS. This may well reflect the age of the patients as the median age in this cohort was 29.58 years (IQR 19.74 to 36.70) compared to median ages in H19/IGF2 LOM and matUPD7 cases of 3.6 years (IQR 1.8-8.4) and 6.4 years (3.6-10.1) in the Wakeling cohort.

A history of reflux was given in 15.2% which is lower prevalence than described in childhood, during which it appears to be a key feature. This may reflect insufficient documentation, recall bias, historically reduced diagnosis of reflux or reduced symptoms of reflux in these individuals.

There appeared to be a high prevalence of recurrent infections and respiratory illnesses; 36.4% compared to an estimated UK prevalence of 20% (292), although the UK estimate only included longterm conditions, which would result in a lower prevalence than found in the studies presented in this thesis. A high prevalence of respiratory disorders may reflect hypotonia and impaired clearance of secretions in childhood resulting in lower respiratory tract infections. Individuals with SRS in this cohort were affected by multiple health problems for which ongoing healthcare was required. This suggests that care for individuals with SRS would impact on the health service – on individual basis although numbers of patients might be lower than for other conditions. Furthermore, the high burden of medical conditions supports the need for long-term follow-up in SRS which has been proposed in the recent international consensus (15).

The clinical scoring systems proposed to-date displayed poor sensitivity in diagnosing adult SRS; 12-60% with immediately available data and 48-76% with additional historical data. The NHCSS diagnosed 16.0% (4/25) cases and a further 32.0% (8/25) cases as ‘possible SRS’ with immediately available data. With the inclusion of historical data, SRS was diagnosed in 40.0% (10/25) and a further 40.0% (10/25) cases as ‘possible SRS’. These sensitivities are all lower than the reported sensitivity of 98% which shows that the scoring system has reduced efficacy in older individuals with SRS. Therefore, there is currently no adequate scoring system for clinically diagnosing SRS in adulthood.

5.9.6 Maternal uniparental disomy for chromosome 14

The phenotype of matUPD14 overlaps with SRS with both conditions causing short stature. In a cohort of individuals with disruption of the imprinted region on 14q32 (secondary to matUPD14, paternal deletion at 14q32 or epimutation at 14q32), 72.7% (16/22) had a NHCSS score of ≥4/6, consistent with a clinical diagnosis of SRS. Of note, the median age at end of the study was 7.5 years (range 1.3 to 21.6 years) and that cohort included predominantly individuals with epimutation at 14q32 (277). It remains unclear whether there are greater or fewer differences in phenotype with increasing age and there may be epigenotype-phenotype differences with that affect the degree of clinical overlap with SRS.

Nevertheless, there are key differences between SRS and matUPD14 with individuals with the latter having higher birth weights, no asymmetry and increased prevalence of special educational needs. The comparison presented in Table 5.7 was undertaken between matUPD14 and H19/IGF2 LOM in order to achieve statistical power. Comparison between matUPD14 and matUPD7 might be informative as the absence of asymmetry and higher prevalence of special educational needs are also seen in matUPD7. Individuals with matUPD14 also appear to have a greater tendency to gain weight and have differing features in dysmorphology such as almond shaped eyes and absence of clinodactyly.

Overall, the clinical features of matUPD14 appear sufficiently different to SRS to remain a separate clinical entity. As new clinical cohorts are described and molecular testing increases, there may be greater clarity regarding the phenotypes of each molecular subgroup.

5.9.7 Limitations of this study

Several limitations of this study result from its retrospective nature. Historical reports from families and individuals would have been affected by memory and recall bias. Medical notes were reviewed but these were not all available; the notes of three individuals had been destroyed after their original hospitals had closed and the notes initially transferred to new NHS Trusts. The data ascertained from hospital notes, where they were available, depended on accurate recording of health professionals.

It is important to note that five cases in this study were also part of the Wakeling cohort published in 2010 therefore some of the reported clinical features will overlap. However, where possible, different SRS cohorts have been reviewed.

5.9.8 Conclusions

Table 5.9 Summary table on key features in adults with SRS versus children with comparison to the general population where possible.

Clinical feature	Results in childhood SRS	Results presented in this thesis	Population data
Height ≤-2 SDS	59.4%	60.6%	2.5% by definition
Feeding/appetite	58-84% prevalence of feeding difficulties	Difficulties with food only reported in four cases	
Relative macrocephaly	70-96% prevalence	57.6%	
Triangular facies	94%	25.8%	
Fifth finger clinodactyly	55-62%	78.8%	1-19.5% (293)
Asymmetry	20-60% prevalence	66.7%	
Respiratory disorders	Sporadic reports	36.4%	20%
Arthralgia	Not described	30.3% overall (44.4% of females; 6.66% of males)	Musculoskeletal conditions in females 25.9%; males 31.8%
University education	Not applicable	40%	42%

In summary, this cohort demonstrates that important aspects of the adult phenotype of SRS include short stature and multiple health conditions. However, the other key features of childhood SRS including early feeding failure, disproportionately large head growth, hypotonia and unusual facial appearance are no longer major features of the disorder in adulthood. The exception is asymmetry, more common in H19/IGF2 LOM subtype and affecting 66.7% of this cohort. The high level of university education, while it may represent ascertainment bias, is interesting as many of the same individuals had early developmental delay. The change in

appetite seen across the cohort from early childhood to adolescence is also of great interest and important for health professionals to acknowledge. This may reflect increased appetite with advancing age in the general population, however no evidence was available to confirm or refute this suggestion. Similarly, to the author's knowledge, data is not available on the general population prevalence of: relative macrocephaly, triangular facies or asymmetry (according to the NHCSS definition).

There are also unexpected medical issues that warrant further investigation, including the overall prevalence of respiratory disorders and arthralgia – particularly affecting females. It is possible that many adults with SRS are not seeking specific medical follow-up and remain undiagnosed. The range of medical conditions reported in this thesis suggests that transition and adult clinics for individuals with SRS would be beneficial, which supports advice given in the international SRS consensus statement (15). Long-term follow-up might allow tailored, specific medical advice for SRS to be developed and shared with patients and their families. However, the potential necessity and benefit require further investigation.

Chapter 6 Height and weight in adolescents and adults with Silver-Russell syndrome and the effects of prior recombinant growth hormone treatment during childhood

6.1 STAARS UK cohort

As detailed in section 4.1, 40 participants were recruited to the STAARS UK cohort. The growth parameters of the cohort were presented in Table 4.1. These were measurements taken at the research study appointment or from the medical notes (in four cases aged <18 years). At the time of the study appointment, GH treatment was ongoing in five cases. In the four cases where final growth data were obtained from subsequent medical notes, GH treatment had then been discontinued.

Although there is clinical overlap between SRS and TS, controversy remains as to whether or not TS is part of a spectrum of SRS. Cases of matUPD14 and 'clinical' SRS cases will be considered separately; excluded from the analysis of GH effects in the STAARS UK cohort and European collaborative cohort. This has been done with the aim of reducing the phenotypic heterogeneity within the analysis cohort to focus on differences that might be attributed to GH treatment in those with a molecular diagnosis of SRS. Excluding the cases of matUPD14 and clinical SRS leaves 33 molecularly confirmed cases for further analysis in this chapter.

6.1.1 Evaluation of age subgroups: <18 years versus ≥18 years

The cohort was analysed in two subgroups with 18 years as the cut point. There were no significant differences in gender or molecular genetic diagnosis (Table 6.1). The median height SDS, weight SDS and BMI SDS of individuals aged <18 years were -1.19, -1.21 and -1.72 respectively. The median height SDS, weight SDS and BMI SDS of individuals aged ≥18 years were -3.13, -1.83 and -0.47 respectively.

Table 6.1 Clinical characteristics of the UK cohort including cases of H19/IGF2 LOM and matUPD7 only. Data on gender, molecular genetic diagnosis and growth hormone treatment are presented as number (column percentage). Data on age are presented as median (full range). Data on growth parameters are presented as median (IQR).

	STAARS UK cohort	Individuals <18 years	Individuals ≥ 18 years	P value
n	33	8	25	
Gender				
Male	15 (45.5)	3 (37.5)	12 (48.0)	0.699
Female	18 (54.5)	5 (62.5)	13 (52.0)	
Age, years	29.58 (13.32-69.71)	14.15 (13.32-16.52)	32.88 (22.03-69.71)	
Molecular genetic diagnosis				
H19/IGF2 LOM	27 (71.8)	5 (62.5)	22 (88.0)	0.137
matUPD7	6 (18.2)	3 (37.5)	3 (12.0)	
Growth parameters				
Height, cm	153.2 (143.8-160.1)	154.2 (139.4-162.9)	150.3 (143.8-160.1)	0.853
Height SDS	-2.67 (-2.83 to -1.07)	-1.19 (-3.82 to -0.51)	-3.13 (-3.83 to -1.31)	0.107
Weight, kg	47.10 (41.65-58.38)	43.83 (31.68-48.26)	55.45 (43.85-63.42)	0.081
Weight SDS	-1.72 (-3.76 to -0.13)	-1.21 (-4.14 to -0.19)	-1.83 (-3.76 to -0.11)	0.951
BMI, kg/m^2	20.5 (17.7-25.5)	17.0 (16.2-19.1)	21.2 (19.1-38.0)	**0.003**
BMI SDS	-0.53 (-1.83 to 1.13)	-1.33 (-1.84 to -0.58)	-0.47 (-1.83 to 1.53)	0.310
Growth hormone treatment				
Yes	23 (69.7)	8 (100)	15 (60.0)	0.071
No	10 (30.3)	0 (0)	10 (40.0)	

Of the subgroup aged <18 years, males and females were compared (Table 6.2). There were no significant differences in age or molecular genetic diagnosis. The median height and SDS in females were 153.0 cm and -1.51 compared to 164.0 cm and -0.76 in males (p=0.036 for height, p=0.393 for height SDS). Weight SDS and BMI SDS in males were -1.22 and -1.33 respectively and were -1.20 and -1.33 in females. Cross tabulation could not be performed for GH treatment as this value was a constant (i.e. all cases were treated).

Table 6.2 Clinical characteristics of individuals aged <18 years with H19/IGF2 LOM and matUPD7 only. Data on gender, molecular genetic diagnosis and growth hormone treatment are presented as number (column percentage). Data on age are presented as median (full range). Data on growth parameters are presented as median (IQR). Where indicated * full range given as IQR could not be calculated.

	Individuals <18 years	Males <18 years	Females <18 years	P value
n	8	3	5	
Gender				
Male	3 (37.5)			
Female	5 (62.5)			
Age, years	14.15 (13.32-17.44)	14.47 (13.83-17.44)	13.83 (13.32-16.89)	0.393
Molecular genetic diagnosis				
H19/IGF2 LOM	5 (62.5)	1 (33.3)	4 (80.0)	0.464
matUPD7	3 (37.5)	2 (66.7)	1 (20.0)	
Growth parameters				
Height, cm	154.2 (139.4-162.9)	164.0 (159.4-164.55)*	153.0 (130.9-154.2)	**0.036**
Height SDS	-1.19 (-3.82 to -0.51)	-0.76 (-1.70 to 0.34)*	-1.51 (-4.69 to -0.65)	0.393
Weight, kg	43.83 (31.68-48.26)	47.10 (42.00-48.65)*	38.50 (26.05-52.30)	0.393
Weight SDS	-1.21 (-4.14 to -0.19)	-1.22 (-2.29 to -0.11)*	-1.20 (-5.03 to -0.08)	1.000
BMI, kg/m^2	17.0 (16.2-19.1)	17.5 (16.53-17.97)*	16.2 (15.1-22.1)	0.786
BMI SDS	-1.33 (-1.84 to -0.58)	-1.33 (-1.38 to -0.53)*	-1.33 (-2.49 to 0.67)	1.000
Growth hormone treatment				
Yes	8 (100)	3 (100)	5 (100)	
No	0 (0)	0 (0)	0 (0)	

In the subgroup aged ≥18 years, males and females were compared (Table 6.3). There were no significant differences in age, molecular genetic diagnosis or growth hormone treatment. The median height of females was 144.7 cm compared to 158.1 cm in males (p=0.01) with median SDS of -3.17 and -2.91 respectively (p=0.936). Median weight SDS was lower in males compared to females; -2.18 vs -0.47 (p=0.06). There was a corresponding difference in median BMI SDS; -1.38 in males vs 0.51 in females (p=0.077).

Table 6.3 Clinical characteristics of individuals aged ≥18 years with H19/IGF2 LOM and matUPD7 only. Data on gender, molecular genetic diagnosis and growth hormone treatment are presented as number (column percentage). Data on age are presented as median (full range). Data on growth parameters are presented as median (IQR).

	Individuals ≥ 18 years	Males ≥ 18 years	Females ≥ 18 years	P value
n	25	12	13	
Gender				
Male	12 (48.0)			
Female	13 (52.0)			
Age, years	32.88 (22.03-69.71)	32.75 (22.03-69.71)	33.01 (24.40-56.85)	1.000
Molecular genetic diagnosis				
H19/IGF2 LOM	22 (88.0)	11 (91.7)	11 (84.6)	1.000
matUPD7	3 (12.0)	1 (8.3)	2 (15.4)	
Growth parameters				
Height, cm	150.3 (143.8-160.1)	158.1 (151.0-170.5)	144.7 (140.7-153.6)	**0.010**
Height SDS	-3.13 (-3.83 to -1.31)	-2.91 (-3.98 to -1.14)	-3.17 (-3.83 to -1.70)	0.936
Weight, kg	55.45 (43.85-63.42)	55.48 (41.88-64.96)	54.65 (44.30-61.12)	1.000
Weight SDS	-1.83 (-3.76 to -0.11)	-2.18 (-5.29 to -0.80)	-0.47 (-2.12 to 0.32)	0.060
BMI, kg/m^2	21.2 (19.1-38.0)	20.1 (18.5-26.5)	23.9 (20.4-28.6)	0.186
BMI SDS	-0.47 (-1.83 to 1.53)	-1.38 (-2.27 to 1.02)	0.51 (-0.78 to 1.73)	0.077
Growth hormone treatment				
Yes	15 (60.0)	9 (75.0)	6 (46.2)	0.226
No	10 (40.0)	3 (25.0)	7 (53.8)	

As discussed in section 3.11.2, the total number of participants recruited to the UK STAARS cohort was insufficient to evaluated differences between those treated with GH and those untreated.

6.2 European collaborative data

As a result of the research collaborations formed, data was available on a further 38 individuals with a molecular diagnosis of SRS. All individuals had completed GH treatment.

6.2.1 STAARS French cohort

Data was available on 17 individuals from France with a molecular diagnosis of SRS. The clinical characteristics of this group are presented in Table 6.4 and Table 6.5. This group was comprised of 4 males and 13 females aged 13.17-28.50 years (median 16.59). H19/IGF2 LOM was diagnosed in 94.% (16/17); matUPD7 in 5.9% (1 case).

The median gestation at birth was 38 weeks with median SDS for birth weight, length, and head circumference of -3.30, -3.80 and -1.02 respectively. The median height SDS of this group was -2.30 with a median BMI SDS of -0.60.

Table 6.4 Clinical characteristics of the overall STAARS European cohort and country cohorts including cases of H19/IGF2 LOM, matUPD7 and IGF2 mutation. Data on gender and molecular genetic diagnosis presented as number (column percentage). Age presented as median (full range). Gestation at birth in completed weeks. Data on birth parameters are presented as median (IQR). P values in bold are statistically significant.

	Whole STAARS cohort	**UK cohort**	**French cohort**	**German cohort**	**P value**
n	71	33	17	21	
Gender					
Male	31 (43.7)	15 (45.5)	4 (23.5)	12 (57.1)	0.126
Female	40 (56.3)	18 (54.5)	13 (76.5)	9 (42.9)	
Age	22.03 (13.17-69.71)	29.58 (13.36-69.71)	16.59 (13.17-28.50)	21.29 (15.07-29.38)	**<0.001**
Molecular genetic diagnosis					
ICR1/H19 LOM	57 (80.3)	27 (81.8)	16 (94.1)	14 (66.7)	
matUPD7	12 (16.9)	6 (18.2)	1 (5.9)	5 (23.8)	0.144
IGF2 mutation	2 (2.8)			2 (9.5)	
Birth parameters					
Gestation at birth	38 (36.0-40.0) (n=70)	38 (37-40) (n=32)	38 (34-39)	38.0 (36.0-40.0)	0.793
Birth weight, g	1800 (1460-2110)	1760 (1459-2149)	1800 (1205-2138)	1840 (1590-2175)	
Birth weight SDS	-3.19 (-4.12 to -2.58) (n=70)	-3.08 (-4.13 to -2.59) (n=32)	-3.30 (-3.85 to -2.43)	-3.07 (-4.22 to -2.53)	0.983
Birth length, cm	42.0 (39.0-45.0) (n=49)	40.6 (40.5-47.0) (n=11)	42.0 (38.0-43.8)	42.0 (39.0-45.5)	
Birth length SDS	-3.59 (-4.92 to -2.41) (n=48)	-3.92 (-5.24 to -0.61) (n=10)	-3.80 (-5.21 to -3.10)	-3.09 (-4.23 to -2.11)	0.239
Birth head circumference, cm	33.0 (32.0-34.5) (n=43)	33.0 (30.0-35.3 (n=9)	33.0 (30.9-34.0) (n=16)	33.5 (31.8-34.5) (n=18)	
Birth head circumference SDS	-0.67 (-1.55 to -0.25) (n=43)	-0.57 (-1.17 to 0.27) (n=9)	-1.02 (-1.87 to -0.35) (n=16)	-0.48 (-1.50 to -0.09) (n=18)	0.357

Table 6.5 Clinical characteristics of overall STAARS European cohort country cohorts including cases of H19/IGF2 LOM, matUPD7 and IGF2 mutation. Data on growth parameters are presented as median (IQR). Data on growth hormone treatment presented as number (column percentage). Age, height SDS at start and end, duration of GH treatment and mean GH dosage presented as median (IQR). Treatment to delay puberty presented as number (column percentage). Age, height SDS at start and end, duration of GnRHa treatment presented as median (IQR). Aromatase inhibitor treatment presented as number (percentage). P values shown for differences across UK, French and German cohorts using the Kruskal-Wallis test for continuous variables and the Fisher's exact test for categorical variables. Those in bold are statistically significant. Full ranges rather than IQR range where indicated with *.

	Whole STAARS cohort	**UK cohort**	**French cohort**	**German cohort**	**P value**
n	71	33	17	21	
Growth parameters					
Target height SDS	-0.06 (-0.83 to 0.52) (n=66)	-0.19 (-0.82 to 0.44) (n=31)	0.10 (-1.00 to .40) (n=15)	-0.05 (-0.40 to 0.60) (n=20)	0.824
Height, cm	154.3 (145.7-162.7)	153.2 (143.8-161.0)	148.8 (142.3-159.9)	158.8 (152.1-169.9)	
Height SDS	-2.23 (-3.38 to -1.15)	-2.67 (-3.83 to -1.12)	-2.30 (-3.60 to -1.73)	-1.58 (-2.68 to -1.14)	0.229
Weight, kg	46.50 (39.50-57.40)	47.40 (41.25-58.38)	41.10 (34.40-46.15)	51.80 (41.15-57.75)	
BMI, kg/m2	19.6 (16.9-23.3)	20.5 (17.7-25.5)	17.7 (16.5-20.2)	18.7 (16.0-21.6)	
BMI SDS	-0.60 (-1.80 to 0.51)	-0.53 (-1.99 to 1.13)	-0.60 (-1.35 to 0.20)	-1.10 (-2.90 to 0.20)	0.800
Growth hormone treatment, n (%)					
Yes	55 (77.5)	23 (69.7)	13 (76.5)	19 (90.5)	0.213
No	16 (22.5)	10 (30.3)	4 (23.5)	2 (9.5)	
Age at start, years	5.80 (4.45-8.98) (n=55)	5.58 (3.33-10.02) (n=23)	6.00 (2.60-9.50) (n=13)	5.77 (5.22-7.70) (n=19)	0.800
Height SDS at start	-3.21 (-4.94 to -2.41) (n=49)	-3.20 (-5.22 to -2.38) (n=20)	-3.55 (-4.65 to -1.72) (n=10)	-3.17 (-4.77 to -2.47) (n=19)	0.992
Age at end, years	14.68 (12.98-15.89) (n=49)	15.00 (13.27-16.50) (n=22)	13.5 (12.65-14.55) (n=10)	15.30 (9.67-16.02) (n=14)	0.453
Height SDS at end	-1.10 (-2.32 to -0.70) (n=39)	-1.10 (-3.06 to -0.61) (n=15)	-1.80 (-3.05 to -1.35) (n=9)	-0.84 (-1.74 to -0.07) (n=12)	0.075
Duration of treatment, years	7.10 (3.96-11.00) (n=48)	8.65 (4.13-11.52) (n=21)	6.05 (3.35-7.95) (n=10)	6.35 (3.77-10.29) (n=14)	0.577
Mean GH dosage, mcg/kg/day	47.74 (34.85-55.98) (n=46)	48.84 (35.72-54.96) (n=19)	35.71 (30.72-49.29) (n=9)	52.48 (34.70-61.66) (n=18)	0.081
Puberty details					
Age of puberty onset in females, years	10.00 (9.14-10.97) (n=30)	10.12 (7.17-11.00) (n=13)	9.90 (9.38-11.10) (n=10)	10.05 (9.17-10.96) (n=7)	
Age of puberty onset in males, years	12.50 (11.10-14.00) (n=25)	12.25 (10.50-13.88) (n=12)	11.00 (9.50-12.00) (n=3)*	13.44 (11.55-14.48) (n=10)	
Treatment to delay puberty					
Yes	20 (28.2)	5 (15.2)	6 (35.3)	9 (42.9)	**0.014**
No	45 (63.4)	27 (81.8)	11 (64.7)	7 (33.3)	
Unknown	6 (8.5)	1 (3.0)	0 (0)	4 (20.0)	
GnRHa analogue, n	19/20	4/5	6/6	9/9	
Cyproterone, n	2/20	2/5	0/6	0/9	
Age at start of GnRHa treatment, years	10.76 (9.64-11.30) (n=19)	8.37 (7.47-10.46) (n=5)	10.80 (10.30-11.90) (n=5)	10.89 (9.70-11.83) (n=9)	0.063
Height SDS at start	-1.21 (-2.55 to -0.41 (n=17)	-0.95 (-2.52 to -0.27) (n=4)	-1.30 (-2.90 to -0.75) (n=5)	-0.86 (-2.65 to -0.22) (n=8)	0.619
Age at end of treatment, years	12.99 (12.19-13.97) (n=18)	11.94 (11.41-12.50) (n=4)	13.20 (12.20-14.70) (n=5)	13.09 (12.45-14.10) (n=9)	0.112
Height SDS at end	-1.04 (-2.01 to -0.53) (n=15)	-1.04 (-2.01 to -0.29) (n=3)*	-1.20 (-3.30 to -0.90) (n=5)	-0.73 (-2.29 to -0.24) (n=7)	0.450
Duration of treatment, years	2.30 (1.79-3.12) (n=18)	2.67 (1.89-3.62) (n=4)	2.30 (1.70-3.10) (n=5)	2.19 (1.58 to 3.10) (n=9)	0.786
Aromatase inhibitor use					
Yes	1 (1.4)	0 (0)	1 (5.9)	0 (0)	0.239
No	70 (98.6)	33 (100)	16 (94.1)	20 (100)	

6.2.2 STAARS German cohort

Data was obtained on 21 individuals from Germany with a molecular diagnosis of SRS. Their clinical characteristics are described in Table 6.4 and Table 6.5. This group was comprised of 12 males and 8 females aged 15.07-29.38 years (median 21.29). H19/IGF2 LOM was diagnosed in 66.7% (14/21); matUPD7 in 23.8% (5/21); and an IGF2 mutation had been diagnosed in 2 cases.

The median gestation at birth was 38.0 weeks with median SDS for birth weight, length, and head circumference of -3.07, -3.09 and -0.48 respectively. The median height SDS of this group was -1.58 with a median BMI SDS of -1.10.

6.2.3 European STAARS cohort

The combined data of 71 cases of SRS comprised 43.7% (31/71) males and 56.3% (40/71) females. There were no significant differences in gender between the UK, French and German cohorts. The median age was 22.03 years with a range of 13.17 to 69.71 years. The overall median height SDS was -2.23 and BMI SDS was -0.60. There were significant differences in age between the three country cohorts ($p<0.001$). Between the cohorts, there were no significant differences in: the proportion of molecular diagnoses, SDS for birth weight, length and head circumference, or SDS for height, weight or BMI.

6.2.4 Growth hormone treatment

6.2.4.1 UK cohort

Of the 33 participants with H19/IGF2 LOM or matUPD7, 69.7% had received GH treatment. The median age at the start of GH treatment was 5.58 years (n=23) and median age at the end of treatment was 15.00 years (n=22) with a median duration of treatment of 8.65 years (n=21). The mean GH dosage was available for 20 individuals; median value 48.84 mcg/kg/day. Median height SDS at the start and end of treatment were -3.20 and -1.10 respectively.

100% of individuals aged <18 years had been treated with growth hormone compared to 60% of those aged ≥18 years ($p=0.071$) which may reflect changes in the management of SRS over time (because younger individuals were treated more recently).

6.2.4.2 French cohort

GH treatment had been received in 76.5% (13/17). Median age at the start of GH treatment was 6.00 years (n=13) and median age at the end of treatment was 13.50 years (n=10). The median

duration of treatment with GH was 6.00 years (n=5). Median height SDS at the start and the end of treatment were -3.55 and -1.80 respectively. The median value for mean GH dosage was 35.71 mcg/kg/day (n=9).

6.2.4.3 German cohort

GH treatment had been received in 90.5% (19/21). Median age at the start of GH treatment was 5.77 years (n=19) and median age at the end of treatment was 15.30 years (n=14). The median duration of treatment was 6.35 years (n=14). Median height SDS at the start and the end of treatment were -3.17 and -0.84 respectively. The median value for mean GH dosage was 52.48 mcg/kg/day (n=18).

6.2.5 Treatment to delay puberty

6.2.5.1 UK cohort

The median age of puberty onset was 10.12 years in females and 12.25 years in males. Treatment to delay puberty had been received in 15.2% (5/33). In four cases, this involved GnRHa treatment; in two cases, cyproterone had been received (one of these individuals had additionally received GnRHa treatment). The median ages for the start and of treatment were 8.37 years (n=5) and 11.94 (n=4) years respectively, with a median duration of treatment of 2.67 years (n=4). The median height SDS at the start and end of treatment were -0.95 (n=4) and -1.04 (n=3) respectively.

6.2.5.2 French cohort

The median age of puberty onset was 9.90 years in females and 11.00 in males. Treatment to delay puberty had been received in 35.3% (6/17). All six cases involved treatment with a gonadotropin releasing hormone analogue (GnRHa). The median ages for the start and end of treatment were 10.80 years and 13.20 years (both n=5) respectively, with a median duration of treatment of 2.30 years (n=5). The median height SDS at the start and end of treatment were -1.30 (n=5) and -1.20 (n=5) respectively.

6.2.5.3 German cohort

The median age of puberty onset was 10.05 years in females and 13.44 in males. Treatment to delay puberty had been received in 42.9 % (9/21). All nine cases involved GnRHa treatment. The median ages for the start and of treatment were 10.89 years and 13.09 years (both n=9) respectively, with a median duration of treatment of 2.19 years (n=9). The median height SDS at the start and end of treatment were -0.86 (n=8) and -0.73 (n=7) respectively.

6.2.6 Aromatase inhibitor treatment

Treatment with an aromatase inhibitor was given to one individual (5.9%) in the French cohort but this intervention was not used in the UK or the German cohorts.

6.2.7 Evaluation of GH effects in the overall European collaborative cohort

In the overall STAARS European cohort, 55 individuals (77.5%) had received GH treatment; 16 (22.5%) were untreated (Table 6.6). The proportions of males and females in each group were not significantly different (p=0.391) however the GH-treated group was significantly younger (p=0.026); median age 21.24 years compared to 28.33 in the GH-untreated group. There was no significant difference in molecular genetic diagnoses (p=0.101). The age at GH start was comparable between the country cohorts (p=0.800) as was the duration of treatment (p=0.577). There was some variation in the median GH dosage (p=0.081) (Table 6.5).

Table 6.6 Clinical characteristics of the whole European STAARS cohort. Demographics and molecular genetic diagnosis data presented as number (percentage). Median age and full age range shown. Growth parameters presented as median (IQR). Where some data unavailable n noted. Significant P values denoted in bold.

	GH untreated	GH treated	P value
n	16	55	
Demographics			
Male, n (%)	5 (31.3)	26 (47.3)	0.391
Female, n (%)	11 (68.8)	29 (52.7)	
Age, years (median, range)	28.33 (13.17-69.71)	21.24 (13.36-56.85)	**0.026**
Age, years (median, IQR)	28.33 (19.73-36.97)	21.24 (16.44-27.36)	
Molecular genetic diagnosis			
ICR1/H19 LOM, n (%)	16 (100)	41 (74.5)	
matUPD7, n (%)		12 (21.8)	0.101
IGF2 mutation		2 (3.6)	
Growth parameters			
Height SDS	-2.74 (-3.36 to -1.13)	-2.22 (-3.66 to -1.16)	0.720
Weight	61.65 (43.76-69.60)	45.20 (38.50-55.45)	
BMI, kg/m2	25.8 (18.9-30.3)	18.5 (16.5-21.1)	
BMI SDS	1.66 (-0.73 to 2.03)	-1.10 (-1.80 to 0.00)	**0.002**
Difference in BWT SDS and final height SDS	0.57 (0.22-0.83) (n=15)	0.84 (0.07-1.78)	0.726
Difference in BL SDS and final height SDS	0.92 (-0.34 to 1.50) (n=10)	1.16 (0.11 to 2.48) (n=38)	0.347
Early height SDS	-2.91 (-3.62 to -2.40) (n=12)	-3.46 (-5.15 to -2.76) (n=53)	0.055
Total height gain	0.53 (-0.13 to 1.37) (n=12)	1.53 (0.80 to 2.52) (n=53)	**0.006**
Distance to target height SDS	2.51 (1.76-3.81)	2.30 (1.55 to 3.01) (n=54)	0.485
Early BMI SDS	-2.78 (-3.29 to -1.33) (n=12)	-2.65 (-3.81 to -1.91) (n=52)	0.323
Total BMI SDS gain	3.58 (1.85 to 5.18) (n=12)	1.95 (0.76 to 2.69) (n=52)	**0.005**
Time since GH discontinuation (years)		9.97 (2.68 to 15.94) (n=42)	

Table 6.7 Clinical characteristics of individuals aged ≥18 years. Demographics and molecular diagnoses presented as number (percentage). Age in years presented as median (IQR). Growth parameters presented as median (IQR). Significant P values denoted in bold. Time since GH end in years.

	GH untreated	GH treated	P value
n	13	33	
Demographics			
Male	4 (30.8)	19 (57.6)	0.189
Female	9 (69.2)	14 (42.4)	
Age	33.01 (25.24 to 38.16)	26.96 (21.36 to 31.81)	0.055
Molecular genetic diagnosis			
ICR1/H19 LOM	13 (100)	25 (75.8)	
matUPD7	0 (0)	6 (18.2)	0.225
IGF2 mutation		2 (6.1)	
Growth parameters			
Height SDS	-2.37 (-3.33 to -1.12)	-2.66 (-3.73 to -1.14)	0.751
weight (median, IQR)	64.53(44.33-72.88)	51.80 (40.55-57.00)	
BMI, kg/m2	28.0 (19.77-32.30)	19.7 (17.66-22.14)	**0.006**
BMI SDS (median, IQR)	1.84 (-1.39 to 2.38)	-0.60 (-1.74 o 0.20)	**0.013**
Difference in BWT SDS and final height SDS	0.52 (0.27-1.54) (n=12)	0.84 (0.11-1.81)	0.830
Early height SDS	-2.88 (-3.44 to -2.04) (n=9)	-3.46 (-5.10 to -2.60) (n=31)	0.130
Total height gain	0.66 (-0.42 to 1.71) (n=9)	1.60 (0.73 to 2.50) (n=31)	0.086
Early BMI SDS	-2.66 (-3.25 to -1.28) (n=9)	-3.10 (-3.97 to -2.12) (n=31)	0.157
Total BMI SDS gain	3.95 (2.08 to 5.63) (n=9)	2.04 (0.89 to 3.25) (n=31)	**0.028**
Time since GH discontinuation (years)		13.67 (9.10 to 17.26) (n=29)	
Puberty delay treatment	11 (100) (n=11)	10 (32.3) (n=31)	

Table 6.8 Clinical characteristics of individuals aged <18 years. Demographics and molecular genetic diagnosis data presented as number (percentage) in GH treated group and number alone in GH untreated. Age in years presented as median (IQR). Growth parameters presented as median (IQR) for GH treated group. Median and full range presented for GH untreated group indicated by*. Significant P values denoted in bold.

	GH untreated	GH treated	P value
n	3	22	
Demographics			
Male	1 (33.3)	7 (31.8)	1.000
Female	2 (66.7)	15 (68.2)	
Age	15.81 (13.17-16.31)*	15.76 (14.97-16.90)	0.446
Molecular genetic diagnosis			
ICR1/H19 LOM	3	16 (72.7)	0.554
matUPD7	0	6 (27.3)	
Growth			
Height	144.8 (144.7-151.5)*	153.5 (147.7-161.2)	
Height SDS	-3.10 (-3.41 to -1.80)*	-1.75 (-2.70 to -1.27)	0.238
Weight	43.00 (36.00-61.00)*	41.15 (35.15-46.65)	
BMI, kg/m2	20.5 (17.70-26.58)*	16.95 (15.85-18.55)	
BMI SDS	0.30 (-0.50 to 1.70)*	-1.20 (-2.04 to -0.47)	**0.046**
Difference in BWT SDS and final height SDS	0.66 (-0.20 to 0.82)*	0.85 (-0.29 to 1.87)	0.663
Difference in BL SDS and final height SDS	0.60 (-0.23 to 0.94)*	1.21 (-0.02 to 3.14) (n=14)	0.509
Early height SDS	-3.30 (-3.73 to -2.30)*	-3.79 (-5.33 to -2.89)	0.446
Total height gain	0.32 (0.20-0.50)*	1.52 (0.87-3.05)	**0.006**
Distance to target height	2.51 (1.90-4.00)*	2.16 (1.86-3.06) (n=21)	0.680
Early BMI SDS	-2.90 (-3.57 to -1.20)*	-2.45 (-3.56 to -1.69) (n=21)	0.870
Total BMI SDS gain	3.20 (0.70-5.27)*	1.33 (0.56 to 2.40) (n=21)	0.234
Puberty delay treatment	2 (100) (n=2)	10 (47.6) (n=21)	0.486
Time since GH discontinuation (years)		2.23 (0.27 to 2.92) (n=13)	

6.2.7.1 GH and height

There was no significant difference in height SDS between the GH-treated group and the GH-untreated group; median height SDS -2.22 and -2.74 respectively (p=0.720) (see Figure 6.1). However, total height gain was greater in the GH-treated group than the GH-untreated group; median 1.53 vs 0.53 (p=0.006) (see Figure 6.2). There was a suggestion that the GH-treated group were shorter at treatment initiation than those untreated; early height SDS -3.46 vs -2.91 (p=0.055). There was no significant difference in distance to target height SDS between the GH-treated and the GH-untreated group; median 2.30 vs 2.51 (p=0.485) (Table 6.6).

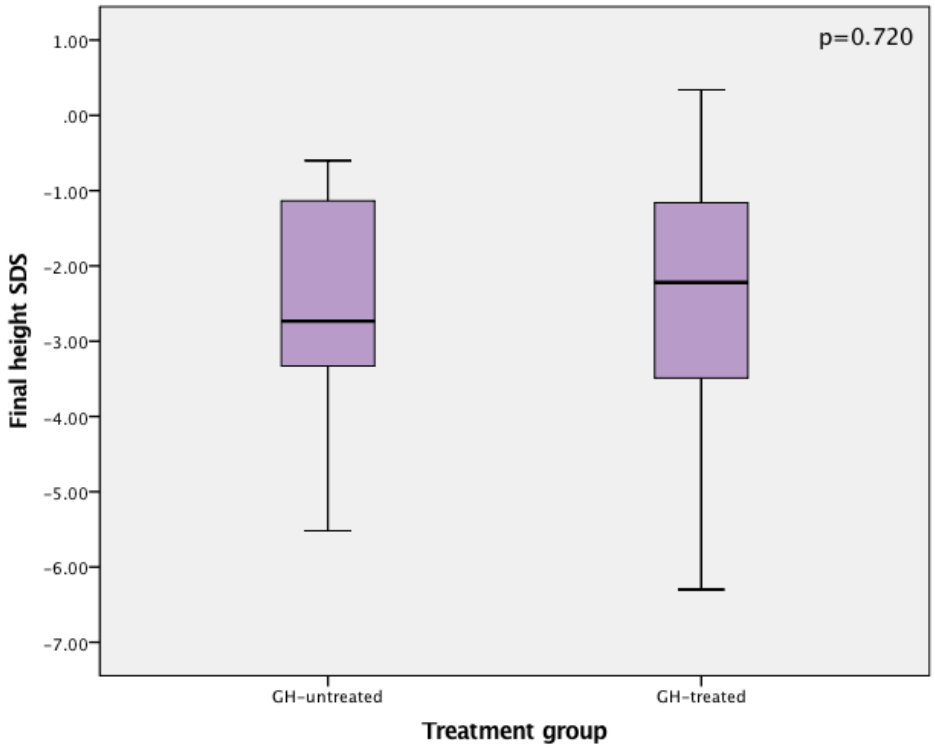


Figure 6.1 Box plots showing final height SDS in GH-untreated and GH-treated groups. P value shown of comparison between the two groups.

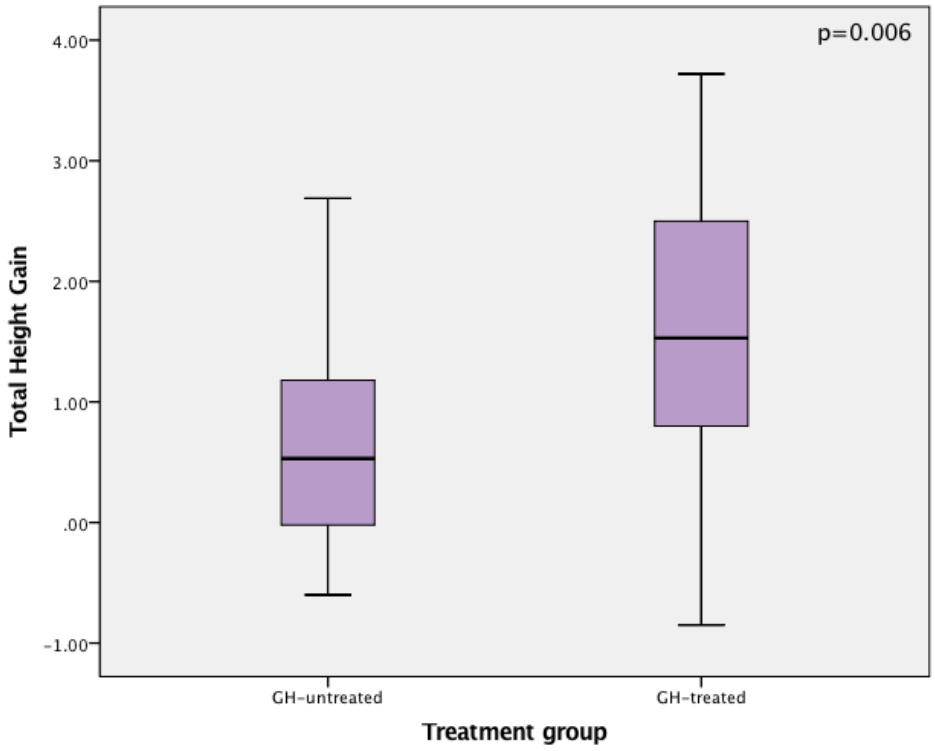


Figure 6.2 Box plots showing total height gain in GH-untreated and GH-treated groups. P value shown of comparison between the two groups.

In individuals aged ≥18 years, there was no difference in height SDS (p=0.751) between the GH treatment groups. Median total height gain in GH-treated and GH-untreated individuals were 1.60 and 0.66 respectively (p=0.086) (Table 6.7).

In individuals aged <18 years, there was no difference in height SDS (p=0.238), however, median total height gain was greater in GH-treated than GH-untreated individuals; 1.52 and 0.32 respectively (p=0.006) (Table 6.8).

6.2.7.2 Growth hormone and weight and BMI

Median weights were 45.20 kg in the GH-treated group and 61.65 kg in the GH-untreated group.

In the overall STAARS European cohort, BMI SDS was lower in the GH-treated group compared to the GH-untreated group; median BMI SDS -1.10 and 1.66 respectively (p=0.002) (see Figure 6.3 and Table 6.6). In the GH-treated group there was a positive correlation between duration of time since GH treatment and BMI SDS; Spearman's rank correlation coefficient 0.341 (p=0.027). In the overall STAARS European cohort BMI SDS positively correlated with age; Spearman's rank correlation coefficient 0.237 (p=0.046).

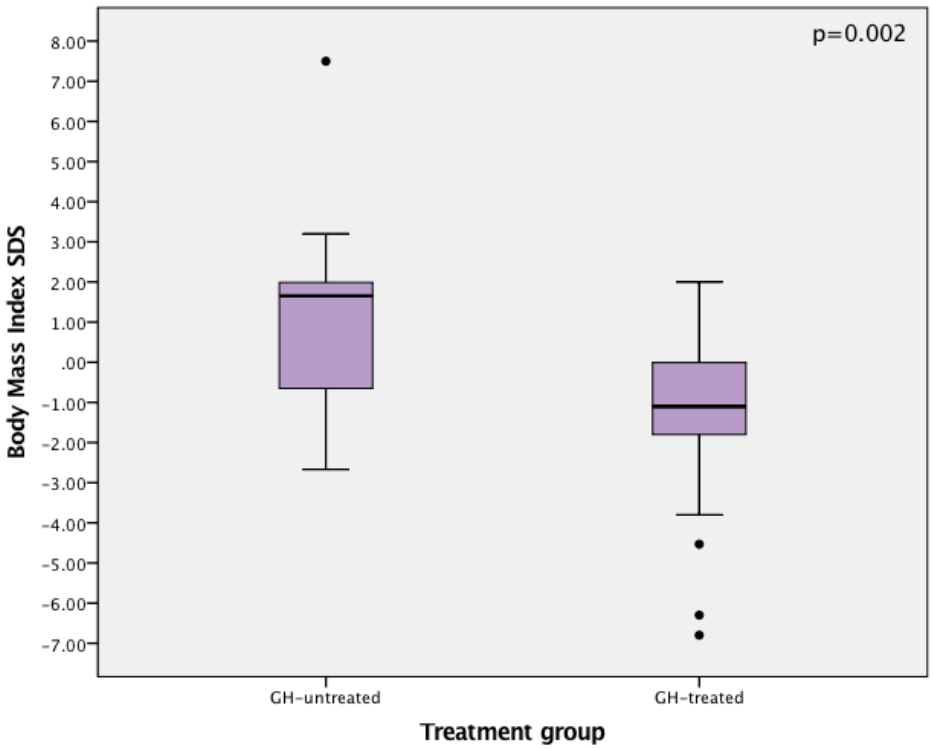


Figure 6.3 Box plots showing body mass index SDS in GH-untreated and GH-treated groups. P value shown of comparison between the two groups.

In individuals aged ≥18 years, median BMI were 19.7kg/m^2 in GH-treated individuals and 28.0 kg/m^2 in GH-untreated (p=0.006). These corresponded to median BMI SDS of -0.60 and 1.84 in GH-treated and GH-untreated individuals respectively (p=0.013). Although early BMI SDS was lower in the GH-treated group than the GH-untreated group; median values -3.10 vs -2.66 (p=0.157), the GH-treated group gained less in BMI; median total BMI gain 2.04 vs 3.95 respectively (p=0.028) (Table 6.7). In the GH-treated individuals aged ≥18 years, there was a positive correlation between duration of time since GH treatment and BMI SDS; Spearman's rank correlation coefficient 0.415 (p=0.025).

In the subgroup <18 years, the median BMI SDS in the GH-treated group and in the GH-untreated group were -1.20 and 0.30 respectively (p=0.046). Median values for total BMI gain in the GH-treated group and in the GH-untreated group were 1.33 and 3.20 respectively (p-0.234) (Table 6.8).

Spearman rank correlations of BMI SDS against age were 0.303 (p=0.087) in GH-treated adults (n=33) and -0.190 (p=0.535) in GH-untreated adults (n=13).

6.2.7.3 Growth hormone and GnRHa and height

Several models for linear regression were performed and included evaluation of the effects of GnRHa. There was no significant impact of GnRHa on final height or total height gain.

6.3 Discussion

6.3.1 Evaluation of the UK, French and German cohorts

6.3.1.1 UK cohort

The description of the UK STAARS cohort compared to previous SRS cohorts has been discussed in Chapter 4 and Chapter 5.

6.3.1.2 French cohort

Unlike other studies (20, 21, 24, 26, 32, 35, 46, 182, 274), the French cohort showed a 3.3:1 female gender preponderance (76.5% vs 23.5%). However, when the country cohorts were compared, the differences did not reach significance (p=0.126). There were 94.1% H19/IGF2 LOM cases compared to 30-60% in other reports (24, 46, 182, 274). Only one individual with matUPD7 was included (5.6%) in the cohort compared to 5-17.2% in other cohorts (24, 46, 182). Owing to the inclusion criteria for the European collaboration, there were no clinical SRS cases in this cohort compared to 31-41% in previous reports (24, 46, 182). The difference in overall molecular

genetic diagnoses (compared to clinical SRS) would be explained by the case ascertainment methods employed, however, this would not account for the difference in prevalence of H19/IGF2 LOM compared to matUPD7. As a result of known (epi)genotype-phenotype correlations in SRS, the predominance of H19/IGF2 LOM cases could affect the research findings. SRS secondary to H19/IGF2 LOM has been shown to be associated with a lower birthweight and reduced catch up growth compared to matUPD7 (21).

6.3.1.3 German cohort

The German cohort shows no significant gender preponderance (42.9% females), similar to other studies (20, 21, 24, 26, 32, 35, 46, 182, 274). In this cohort, there were 66.7% H19/IGF2 LOM compared to 30-60% in previous reports (24, 46, 182, 274). Individuals with matUPD7 comprised 23.8% of the cohort compared to 5-17.2% in other cohorts (24, 46, 182). Two cases of *IGF2* mutation were included. The inclusion of *IGF2* mutations (causing reduced expression of IGF2) as a molecular cause of SRS has been proposed (131, 294) and is supported by international consensus (15). Again, owing to the inclusion/exclusion criteria for the European collaboration, there were no clinical SRS cases in this cohort compared to 31-41% in previous reports (24, 46, 182). The difference in molecular genetic diagnoses would clearly have been affected by the ascertainment methods employed here.

The birth weight SDS of -3.07 seen in this cohort is within the range reported in previous studies which have varied from -2.79 to -3.50 (24, 46, 182, 274). The birth length SDS of -3.09 is comparable to other cohorts reporting -3.01 to 4.13 (24, 46, 182, 274). The head circumference at birth SDS of -1.04 is within the range of previous studies of -1.5 to -0.62 (24)(182). This shows that the birth parameters observed in this cohort are representative of SRS.

6.3.1.4 Comparison of the cohorts from UK, France and Germany

Comparison of the different cohorts, in terms of birth parameters (i.e. gestational age at birth, birth weight SDS, birth length SDS, birth head circumference SDS) and growth parameters (i.e. height SDS and BMI SDS) did not demonstrate significant differences. There were no significant differences in: age and height at start of GH treatment; age and height at end of GH treatment; duration of GH treatment; age and height at start of GnRHa treatment; age and height at end of GnRHa; duration of GnRHa treatment; and target height SDS. However, there were significant differences in the numbers of cases treated to delay puberty ($p=0.014$). There were suggestions of differences in height SDS at end of GH treatment, mean GH dosage, and age at start of GnRHa treatment between the three country cohorts; $p=0.075$, $p=0.081$ and 0.063 respectively.

6.3.1.5 European cohort

The birth weight SDS of -3.19 seen in the overall cohort is similar to previous studies which have ranged from -2.79 to -3.50 (24, 46, 182, 274). The birth length SDS of -3.59 is within the range of other reports which have found values to be -3.01 to 4.13 (24, 46, 182, 274). The head circumference at birth SDS of -0.67 is near the upper limit of previous studies of -1.5 to -0.62 (24)(182). Overall, the birth parameters observed in this cohort are representative of SRS. Many of the individuals included in the overall cohort presented here would have been included in previous SRS cohorts therefore there is some overlap in the reported data.

6.3.2 Effects of prior GH treatment on height in SRS

In this cohort of 71 individuals, previous GH treatment was not associated with increased height SDS. This is contrary to the only other study of final height in adults with SRS, which found a higher mean adult height SDS of -2.12 in patients treated with GH compared to -3.13 in those untreated (46). By comparison, in this study median height SDS in the GH-treated individuals was similar at -2.22 but the GH-untreated group was taller with a median height SDS of -2.74.

GH treatment was, however, associated with a greater total height gain reflecting the shorter early heights in the GH-treated group and suggesting that the decision to treat with GH was made in shorter children with SRS. GH-treated individuals were younger than GH-untreated. This may reflect changes in clinical practice such that GH treatment is now more frequently given in SRS, making it more unlikely to find younger GH-untreated individuals.

Some individuals reported in the study by Binder et al. (46) would also be included in the European STAARS cohort. However, the previous study included 29 'clinical' SRS cases out of 50, which may have influenced the conclusions. These 29 cases were not included in this research, therefore the maximum number of possible overlapping cases is 21. 21 cases were included in this work however 2 had *IGF2* mutations and were not included in the previous report. The median height SDS in the GH-treated individuals of -2.22 is similar to another report of final/adult height SDS; -2.17 (182) suggesting that height attained in the European cohort is comparable to other centres.

A dose-dependent relationship has been demonstrated between GH and height velocity in studies of children born SGA (169, 295) therefore it is plausible that the difference in GH dosage between the country cohorts, could affect the height SDS attained at the end of treatment. However, in another study mean GH dosage has not been associated with height outcome in SRS (46).

6.3.3 Effects of prior GnRHa treatment on height in SRS

Although the reported ages of puberty onset were similar across the different cohorts, the numbers of individuals treated to delay puberty was significantly different (p=0.014). The highest rate of treatment was observed in the German cohort and the lowest in the UK cohort. GnRHa treatment has been used since the 1980s to block production of sex steroids and therefore reduce their effects on bone maturation in an attempt to prolong linear growth. GnRHa treatment is recommended in precocious puberty (i.e. onset of puberty at age <8 years in girls and <9 years in boys) where there is rapid progression in pubertal development, however routine use is neither recommended in children born SGA nor in idiopathic short stature (296). Combination treatment with 2 years of GnRHa therapy in addition to GH has been shown to benefit children with short stature who were born SGA (297, 298). Between the country cohorts, there were no significant differences between the height SDS at the start or end of treatment to delay puberty, which might suggest that similar treatment indications were applied. In the European cohort described here the duration of treatment was comparable to the international recommendation of at least 2 years (15) and there were no differences in the duration of this treatment between the country cohorts. However, in this cohort there was no observed significant effect of GnRHa treatment. This may have resulted from the small number of individuals treated (19/71) or it may reflect variation in treatment with older individuals in this study and possible historical differences in treatment regimens.

6.3.4 Effects of prior GH treatment on body mass index in SRS

In the present study, GH treatment was associated with a lower BMI SDS. The observed difference was greatest in the overall cohort (median BMI SDS 1.66 in the GH-untreated group; -1.10 in the GH-treated group) although this included individuals aged <18 years, some of whom had very recently stopped GH treatment. In individuals aged ≥18 years, the observed median BMI SDS were 1.84 in the GH-untreated group and -0.60 in the GH-treated group. In individuals aged <18 years the median BMI SDS were 0.30 in the GH-untreated group and -1.20 in the GH-treated group. In SGA, GH treatment has been demonstrated to reduce fat mass and promote the development of lean mass during treatment (299). In contrast, one study has shown increased fat mass during and two years after GH treatment in SRS (80). BMI SDS increased with greater time since GH discontinuation and also with increasing age. However, the correlation was stronger with time since GH discontinuation.

Absolute BMI was appropriate for evaluation in individuals aged ≥18 years and showed that the lower quartile cut points were 19.77 kg/m^2 in the GH-untreated group and 17.66 kg/m^2 in the treated group which suggests that the GH-treated group showed more of a tendency to BMI

below 18.5 kg/m^2 which is classified as underweight (236). The upper quartile cut points were 32.30 kg/m^2 in the GH-untreated group and 22.14 kg/m^2 in the GH-treated group showing that the there was also a tendency to BMI above 30 kg/m^2 in the GH-untreated group, which is classified as obese (236). Children with SRS are frequently described as 'underweight' and this is usually associated with feeding difficulties. However, neither weight status nor ongoing feeding difficulties have been reported in adulthood in SRS.

Body composition in SRS has been evaluated in a study of seven patients with SRS. BMI SDS ranged from -2.8 to 2.5 (BMI 16.3-32.3 kg/m^2). These individuals were younger than those reported here, GH had been received in 2/7 cases and treatment effects were not analysed (52). To the author's knowledge, long-term BMI after GH treatment has not been reported in SRS.

Lower BMI in GH-treated individuals may result from increased muscle mass and/or reduced fat mass. Previous GH treatment could promote the development of muscle mass and reduction of fat mass as a direct action. However, it is unclear whether these changes would persist several years following treatment discontinuation. In a study by Smeets et al. of 29 individuals with clinically and molecularly confirmed SRS, treated with 35 mcg/kg/day GH, lean body mass reduced on treatment, reduced further in the first six months following treatment cessation and then stabilised. In the same study fat mass percentage increased during GH treatment, increased further following the end of treatment and then stabilised (80). The change in body composition reported by Smeets et al. (80) does not support the theory postulated in this thesis and contrasts with previous reports of reduced body fat and increased lean mass during GH treatment in SGA. As discussed in section 1.13.3.1, GH treatment in SGA has been associated with: 1) reduced skin fold thickness (184) which have been reported to subsequently increase on treatment (185); 2) reduced skin fold thickness without a change in fat mass but accompanied by increased lean mass (172); and 3) increased lean mass during the first year of treatment followed by a return to baseline over the next two years of treatment (64). In SGA, six months after GH treatment discontinuation, increased body fat percentage, fat mass and decreased lean body mass have been reported (187).

One study reported body composition in 59 adults who were born SGA and previously treated with GH and found that fat mass, fat distribution and lean body mass were comparable to 52 untreated SGA adults. BMI SDS were comparable between the two groups; 0.3 (SD 1.2) and 0.3 years (SD 1.6) respectively (66). The GH-treated group was younger than the GH-untreated group; mean ages 22.5 years and 20.9 years respectively. The GH-treated group was also taller; mean height SDS -1.6 vs -2.5. GH treatment had been given for a mean of 7.7 years (SD 2.4) and discontinued for a mean of 6.8 years (SD 1.8). Duration of treatment was not associated with fat mass or lean body mass. Compared to the SGA adults, the individuals included in the studies

presented in this thesis were older, the duration of GH treatment was similar (median 7.1 years) and the duration of time since GH treatment cessation was greater (median of 9.97 years). However, the GH-untreated group presented in this thesis was shorter than the SGA adults. Overall, the study of SGA adults does not support the idea of sustained changes in body composition following GH treatment.

Alternatively, GH treatment could enable individuals to have increased vitality during treatment and therefore be associated with increased exertion. Subsequently there might be advantageous habits (e.g. regular exercise) and development of muscle mass and reduction of fat mass as a secondary effect.

6.3.5 Limitations

There may be limitations to the use and interpretation of BMI at the extremes of stature. A previous study in healthy children (unaffected by SRS) reported a tendency for body mass index to be lower in shorter children and higher in taller children and the authors suggested using BMI-for-height-age rather than BMI-for-age in children aged 10-14 years with short stature (300). The use of body mass index to detect obesity-associated medical problems has been criticised in adults with short stature in a Mexican study and that study proposed a lower threshold of 25 kg/m^2 should be considered obese. The authors also highlighted the exponential relationship between weight and height in the calculation of BMI, which may lead to discrepancies at the extremes of either variable (301). High body fat composition has been reported in short stature compared to tall stature (302). Although the latter study was also conducted in Mexico, the findings in short stature could be applicable to short stature seen in SRS, however there may be unknown confounding factors. Furthermore, although there may be limitations to the interpretation of BMI, the GH-treated and GH-untreated in the European STAARS cohort did not have significantly different heights yet significantly different BMI were observed. Discussion of body composition, which was also evaluated as part of this research will be included in Chapter 7.

There are weaknesses associated with the analysis of the combined UK, French and German collaborative cohort. Although the proportion of males and females in the GH treatment groups was similar, information on the ethnicity and socio-economic status of individuals with SRS was unavailable. Consequently, other differences between the groups cannot be excluded. The GH-untreated group was significantly older than the GH-treated group therefore the two groups may have been eligible for different treatments. Patients were treated at different historical time points over long time period and medical practice is likely to have changed and developed in each country over this period. Data was also collected at different times. The rationale for treatment could not be confirmed although all individuals had molecularly confirmed SRS therefore the

indication of SGA without catch-up growth could be inferred. The circumstances prior to GH treatment were unknown. For example, nutritional intervention(s) and growth pattern, including weight gain, before starting GH were unknown.

The median GH dosage was also above the current treatment recommendation and there was a suggestion of variation in GH dosage between the UK, French and German cohorts. Between the countries, there were differences in the addition of treatment to delay puberty. The reasoning behind decisions to delay puberty were not available and therefore no consistent approach to treatment could be applied in our study. Treatment to delay puberty was not associated with final height SDS or total height gain in this study and therefore this is unlikely to have affected the results. This is in contrast to previous studies in children with short stature who were born SGA, which showed improved adult height with combination treatment with 2 years of GnRHa therapy in addition to GH (297) and greater height gain (298).

Finally, other potential unmeasured confounding factors could not be examined. Advice given by treating teams regarding exercise and other lifestyle modifications in SRS is unknown. For example, there may have been differences between medical teams who administered GH treatment and those which did not. This may have led to better advice about avoiding weight gain in participants who were born SGA.

The limitations of this retrospective, observational study, including data from multiple countries over a prolonged time period, highlight the importance of randomised controlled trials. It is worth noting that the UK National Institute for Health and Care Excellence reached the following conclusion regarding the evidence on GH treatment in SGA: 'The Assessment Group considered the studies to be generally of poor methodological quality'. If it were possible to investigate anew the effectiveness of GH in SGA, a research study could be designed to prospectively evaluate differences in treatment groups (after ensuring the necessary research and ethics approvals were obtained). It would be ideal to define the research question and hypothesis/hypotheses ahead of the study start date. Such a study would, ideally, be designed to recruit treatment groups matched for age, sex ethnicity, socio-economic status, target height SDS and birth weight SDS. Study endpoints would be pre-determined and include: gain in height SDS, adult height SDS, body composition and quality of life. Treatment arms would include: GH treatment with GnRHa, GH treatment alone, and no GH with no GnRHa. Treatment would be standardised, including GH dosing and regimen, and indications for the addition of GnRHa. It would be difficult to guarantee similarities in the underlying aetiology of SGA and it would not be able to include exclusively SRS unless tested early and likely would take a number years to recruit adequate numbers. Would not be able to consider future treatments or newly observed effects/associations of GH treatment. It

would be important to perform interim analysis so that if one group was showing clear treatment benefits, the planned treatment groups.

6.3.6 Conclusions

Although GH treatment was not associated with increased adult height (or near adult height) in this SRS cohort, this may have been because the decision to treat was made in more severe short stature as there was significant height gain in those treated compared to those untreated.

A novel association of reduced BMI with previous GH treatment has been described in older individuals with SRS. Of note, the GH-treated individuals were younger and BMI SDS was also shown to increase with age. However, the lower BMI SDS was in GH-treated individuals compared to GH-untreated individuals and was seen in the cohort overall, those aged ≥18 years and those aged <18 years.

These findings may suggest alternative or additional benefits to GH treatment in SRS during childhood with long-term benefit to optimise body composition several years after cessation of GH treatment.

Chapter 7 Body composition, metabolic health and the effects of prior growth hormone treatment in SRS

7.1 Introduction

This chapter will report the results of the 33 individuals with molecularly confirmed SRS in the STAARS UK cohort. These were 15 males and 18 females with a median age of 29.58 years (range 13.36 to 69.71). 23 were in the GH-treated group and 10 were GH-untreated. All individuals aged <18 years were treated with GH and at the time of the study appointment, four were receiving recombinant GH therapy at the time of assessment.

7.2 Anthropometry

7.2.1 Body mass index and weight categories in SRS

In Chapter 6 the whole European STAARS cohort was discussed and BMI SDS was demonstrated to be lower in GH-treated vs GH-untreated individuals. By contrast, in the 33 individuals in the UK STAARS cohort the median BMI SDS was -0.53 and although median BMI SDS was lower in the 23 GH-treated individuals than the 10 GH-untreated individuals (-1.09 (IQR -1.67 to 0.51) versus 0.57 (IQR -2.07 to 2.22)), this did not reach statistical significance (p=0.155). This chapter will include evaluation of correlations with BMI SDS as these may suggest the reason(s) for differences in BMI observed between GH treatment groups in the whole European STAARS cohort.

Weight categories were assigned using the WHO classification as described in section 3.6.2. In individuals aged ≥18 years (n=25) weight categories were: underweight (BMI <18.5 kg/m^2) in 20%, ideal weight (BMI 18.5-24.99 kg/m^2) in 44%, overweight (BMI 25 kg/m^2 to 29.99 kg/m^2) in 24%, obese class I (BMI 30 kg/m^2 to 34.99 kg/m^2) in 8%, and obese class II (BMI ≥35 kg/m^2) in 4%. Figure 7.1 shows the proportions of each weight category in the GH-untreated and GH-treated groups of individuals aged ≥18 years. Obesity was seen only in the GH-untreated group; 30% vs 0% of GH-treated group (p=0.052).

The prevalence of weight categories in individuals aged <18 years (n=8) were: underweight in 62.5%, ideal weight in 25.0%, overweight in 12.5%. There were no GH-untreated individuals in this age group. There was a suggestion of difference in spread of weight categories between individuals aged ≥18 years and those aged <18 years (p=0.055).

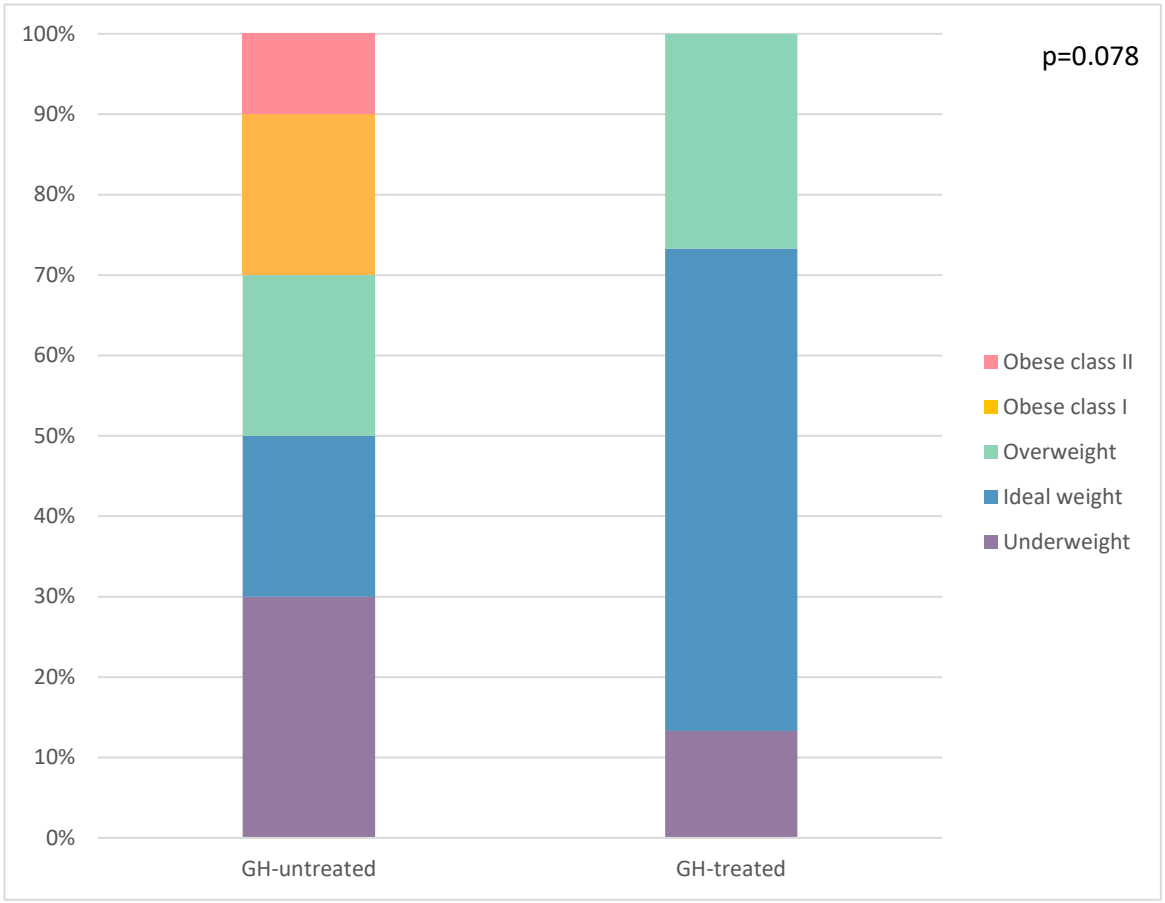


Figure 7.1 Weight categories stratified by GH treatment group. Data presented as a percentage of GH-untreated (n=10) and GH-treated (n=15) in individuals aged ≥18 years. Underweight, BMI <18.5. Ideal weight, BMI ≥18.5 to <25. Overweight, BMI ≥25 kg/m^2 to <30 kg/m^2. Obese class I, BMI ≥30 kg/m^2 to <35 kg/m^2. Obese class II, BMI ≥35 kg/m^2). Median ages of GH-untreated and GH-treated groups were 35.2 years (range 24.4-69.7) and 32.4 years (range 22.0-56.9) respectively. P value shown for comparison of GH-untreated versus GH-treated groups.

7.2.2 Waist-to-hip ratios in SRS

Waist-to-hip ratios were calculated as described in section 3.6.2. The mean in males aged ≥18 years (n=12) was 0.928 (SD 0.055). GH-untreated (n=3) and GH-treated (n=9) individuals in this subgroup had mean waist-to-hip ratios of 0.929 (SD 0.048) and 0.927 (SD 0.060) respectively (p=0.953). The mean waist-to-hip ratio in females aged ≥18 years (n=13) was 0.830 (SD 0.070). GH-untreated (n=7) and GH-treated (n=6) individuals in this subgroup had mean waist-to-hip ratios of 0.832 (SD 0.075) and 0.827 (SD 0.071) respectively (p=0.894).

The median waist-to-hip ratio in males aged <18 years (n=3) was 0.790 (full range 0.769 to 0.830). The median waist-to-hip ratio in females aged <18 years (n=5) was 0.796 (IQR 0.754 to 0.875). All individuals aged <18 years were GH-treated. There was a significant difference in waist-to-hip ratio when comparing those aged ≥18 years and those aged <18 years (p=0.036).

In the cohort overall, 30.3% (10/33) had an elevated waist circumference; ≥80 cm in females and ≥94 cm in males. Including only individuals aged ≥18 years the prevalence of a high waist circumference was 36.0% (9/25). One individual aged <18 years (12.5%, n=8) had a high waist circumference.

7.3 Body composition in SRS

Body composition was assessed by assessing three components: fat mass, lean mass and bone mass. Three methods were used to assess fat mass: skinfold thickness measurements (n=30), bioelectric impedance analysis (BIA) (n=32) and dual-energy x-ray absorptiometry (DXA) (n=22). The equations used to calculate percentage body fat from skinfold thicknesses were described in section 3.7.5. The output from BIA was displayed on the Bodystat Quadscan 400 and transferred to the research notes manually. Figure 7.2 shows an example of the printed output of DXA body composition analysis. The electronic output included the full analysis data.

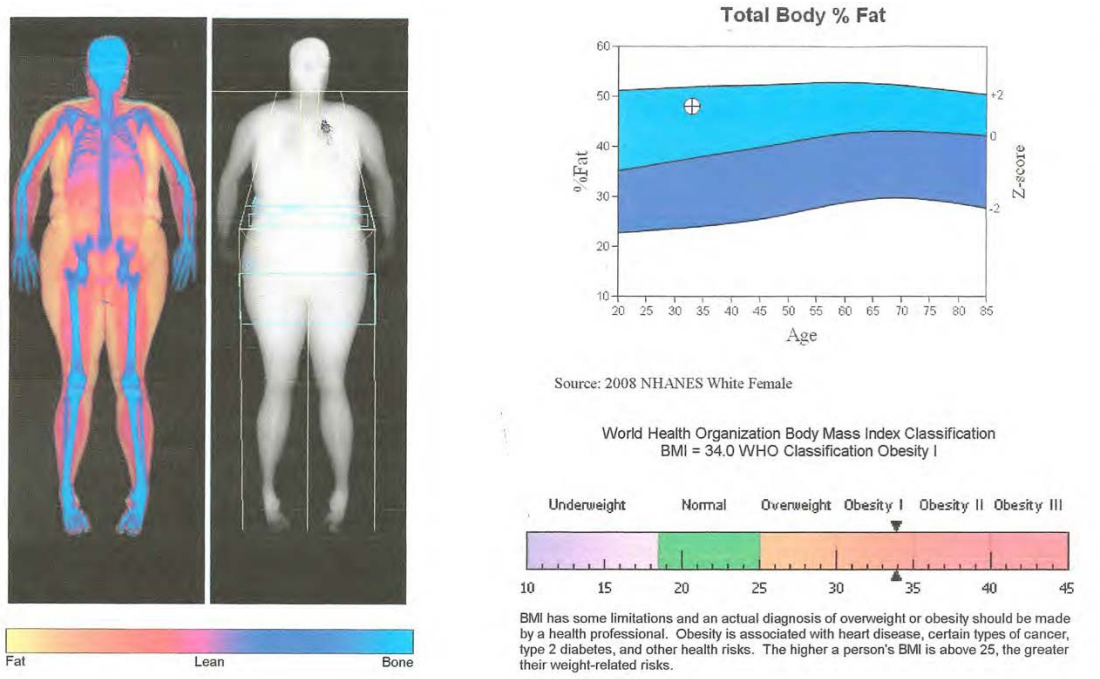


Figure 7.2 An example of the output of the Hologic DXA body composition analysis. This result was obtained from a STAARS participant (Case ID 27).

7.3.1 Fat mass in SRS

Body fat percentage was calculated from SFTs, BIA and DXA. Overall median body fat percentage was calculated as 20.79%, 29.95% and 41.31% respectively (Table 7.1). In individuals aged ≥18 years median body fat percentage was calculated as 24.25%, 31.75% and 44.45% respectively (Table 7.2). In individuals aged <18 years median body fat percentage was calculated as 16.39%, 22.85%, and 29.10% respectively (Table 7.3). The calculations of fat mass using skin fold thicknesses do not include height data but do adjust for age and sex. It is possible that the same measurements in a taller individual (of the same age and sex) would reflect a lower body fat estimate. BIA and DXA calculations do include height and weight factors, however with low heights and weights of individuals in this study, the accuracy of the calculations may be questioned.

Median body fat percentage calculated from SFTs was greater in individuals aged ≥18 years compared to those aged <18 years; 24.25% and 16.39% respectively (p=0.045). Median BIA fat mass index (FMI) was significantly greater in individuals aged ≥18 years than in those aged <18 years; 6.61 vs 3.87 (p=0.029). Median DXA body fat percentage was 44.45% in individuals aged ≥18 years and 29.10% in individuals <18 years old (p=0.300). All fat parameters were higher in individuals ≥18 years than in individuals <18 years old (Table 7.3). Fat mass and body fat percentage have been shown to increase with age (303, 304).

Overall BMI SDS correlated with body fat percentage from SFTs (Spearman's rank correlation coefficient 0.685, p<0.001), BIA body fat percentage (Spearman's rank correlation coefficient 0.353, p=0.048), BIA FMI (Spearman's rank correlation coefficient 0.636, p<0.001) as well as DXA body fat percentage (Spearman's rank correlation coefficient 0.678, p=0.001), and DXA FMI Spearman's rank correlation coefficient 0.648, p=0.001). In individuals ≥18 years, BMI SDS correlated with body fat percentage from SFTs (Spearman's rank correlation coefficient 0.721, p<0.001), BIA FMI (Spearman's rank correlation coefficient 0.709, p<0.001) as well as DXA body fat percentage (Spearman's rank correlation coefficient 0.690, p=0.002), and DXA FMI Spearman's rank correlation coefficient 0.667, p=0.002).

7.3.2 Fat mass and the effects of GH in SRS

Body fat percentage calculated from SFTs and BIA fat mass index were significantly lower in GH-treated individuals compared to GH-untreated individuals (Table 7.1). BIA fat percentage, DXA fat percentage (total and subtotal) and DXA fat mass index (FMI) were all lower in GH-treated individuals but did not reach statistical significance (Table 7.1). However, fat mass is known to

increase with age (303, 304) and when individuals aged <18 years were excluded from analysis, there were no significant differences in body fat between the GH treatment groups (Table 7.2).

Table 7.1 Body composition results from SFTs, BIA and DXA displayed for the STAARS UK cohort separated by GH treatment group. Results for male/female are number (percentage). Age presented as median (full range). All other results shown as median (interquartile range). n=number. ^n=13 *n=21

	All	**GH-untreated individuals**	**GH-treated individuals**	**P value**
	n=33	n=10	n=23	
Male/female	15 (45.5%)/18 (54.5%)	3 (30%)/7 (70%)	12 (52.2%)/11 (47.8%)	0.283
Age	29.58 (13.36-69.71)	35.17 (24.40 to 69.71)	26.99 (13.36 to 56.85)	**0.020**
	n=30	n=8	n=22	
SFT body fat percentage	20.79 (16.17 to 27.90)	26.45 (20.98 to 33.85)	17.79 (15.06 to 26.38)	**0.040**
	n=32	n=10	n=22	
BIA fat percentage	29.95 (19.95 to 38.20)	35.05 (29.33-40.38)	26.20 (18.55 to 35.23)	0.058
BIA fat mass index	5.99 (3.50 to 8.94)	7.98 (5.33 to 12.18)	5.24 (3.18 to 7.50)	**0.028**
BIA fat-free mass index	14.73 (12.43 to 17.81)	17.41 (12.39 to 19.41)	14.52 (12.33 to 16.40)	0.235
	n=22	n=8	n=14	
DXA total fat percentage	41.31 (29.53 to 46.88)	44.48 (33.43 to 47.66)	35.47 (25.95 to 46.53)	0.402
DXA subtotal fat percentage	42.77 (29.83 to 48.41)	46.11 (34.31 to 48.96)	36.24 (26.05 to 48.58)	0.525
DXA fat mass index	8.03 (4.45 to 13.10)	11.42 (5.92 to 14.72)	6.63 (4.03 to 11.83)	0.095
DXA fat-free mass index	13.02 (11.65 to 14.88)	14.80 (11.68 to 17.05)	12.91 (11.52 to 13.69)	0.212
DXA lean mass index	12.45 (11.02 to 14.10)	14.01 (11.03 to 16.20)	12.25 (10.89 to 13.02)	0.212
DXA %fat trunk /% fat legs	1.01 (0.88 to 1.13)*	0.99 (0.87 to 1.17)	1.01 (0.80 to 1.13)^	0.804
DXA trunk/limb fat ratio	1.16 (0.92 to 1.35)*	1.18 (0.80 to 1.34)	1.11 (0.94 to 1.35)^	0.645

Table 7.2 Body composition results from SFTs, BIA and DXA displayed for individuals aged ≥18 years in the STAARS UK cohort separated by GH treatment group. Results show median (interquartile range).

	Individuals aged ≥18 years	**GH-untreated individuals aged ≥18 years**	**GH-treated individuals aged ≥18 years**	**P value**
	n=22	n=8	n=14	
SFT body fat percentage	24.25 (17.33 to 28.70)	26.45 (20.98 to 33.85)	21.30 (15.90 to 27.90)	0.188
	n=24	n=10	n=14	
BIA fat percentage	31.75 (21.70 to 38.88)	35.05 (29.33 to 40.38)	30.40 (19.53 to 36.03)	0.138
BIA fat mass index	6.61 (4.50 to 9.16)	7.98 (5.33 to 12.18)	6.26 (3.61 to 8.23)	0.138
BIA fat-free mass index	16.27 (12.71 to 18.33)	17.41 (12.39 to 19.41)	15.73 (12.76 to 17.93)	0.625
	n=18	n=8	n=10	
DXA total fat percentage	44.45 (31.45 to 46.88)	44.48 (33.43 to 47.66)	40.66 (29.53 to 46.53)	0.573
DXA subtotal fat percentage	46.09 (32.03 to 48.42)	46.11 (34.31 to 48.96)	41.99 (29.83 to 48.58)	0.762
DXA fat mass index	9.33 (5.29 to 13.53)	11.42 (5.92 to 14.72)	7.90 (3.10 to 11.83)	0.173
DXA fat-free mass index	13.13 (11.31 to 15.64)	14.80 (11.68 to 17.05)	12.46 (8.18 to 14.07)	0.237
DXA lean mass index	12.38 (10.71 to 14.90)	14.01 (11.03 to 16.20)	11.71 (7.74 to 13.31)	0.237
DXA %fat trunk /% fat legs	1.02 (0.88 to 1.16)	0.99 (0.87 to 1.17)	1.05 (0.90 to 1.18)	0.573
DXA trunk/limb fat ratio	1.21 (0.94 to 1.36)	1.18 (0.80 to 1.34)	1.21 (0.98 to 1.49)	0.408

Table 7.3 Body composition results from SFTs, BIA and DXA displayed by age subgroup. Results show median (interquartile range) except for age where median age and full range given. n=number. * n=3 and full data range given.

	Individuals aged ≥18 years	**Individuals aged <18 years**	**P value**
	n=25	n=8	
Male/female	12 (48.0%)/13 (52.0%)	3 (37.5%)/5 (62.5%)	
Age	32.88 (22.03-69.71)	15.04 (13.36 to 17.44)	
	n=22	n=8	
SFT body fat percentage	24.25 (17.33 to 28.70)	16.39 (14.86 to 20.05)	**0.045**
	n=24	n=8	
BIA fat percentage	31.75 (21.70 to 38.88)	22.85 (15.58 to 31.90)	0.135
BIA fat mass index	6.61 (4.50 to 9.16)	3.87 (2.59 to 5.34)	**0.029**
BIA fat-free mass index	16.27 (12.71 to 18.33)	14.36 (11.46 to 14.53)	**0.033**
	n=18	n=4	
DXA total fat percentage	44.45 (31.45 to 46.88)	29.10 (22.67 to 47.94)	0.300
DXA subtotal fat percentage	46.09 (32.03 to 48.42)	29.52 (22.26 to 49.71)	0.300
DXA fat mass index	9.33 (5.29 to 13.53)	5.53 (3.87 to 11.41)	0.434
DXA fat-free mass index	13.13 (11.31 to 15.64)	13.02 (12.04 to 13.48)	0.837
DXA lean mass index	12.38 (10.71 to 14.90)	12.45 (11.41 to 12.87)	0.837
DXA %fat trunk /% fat legs	1.02 (0.88 to 1.16)	0.92 (0.79 to 10.6)*	0.356
DXA trunk/limb fat ratio	1.21 (0.94 to 1.36)	0.94 (0.90 to 1.11)*	0.221

In the cohort overall, time since GH discontinuation positively correlated with SFT body fat percentage; Spearman's rank correlation coefficient 0.407, p=0.067, BIA fat percentage; Spearman's rank correlation coefficient 0.427, p=0.053, and BIA FMI; Spearman's rank correlation coefficient 0.516, p=0.017 (see Figure 7.3). BIA FMI correlated more positively with age (than with time since GH discontinuation); Spearman's rank correlation coefficient 0.576, p=0.001 (see Figure 7.4). There were no significant correlations between time since GH discontinuation and either DXA total fat percentage or DXA FMI.

In individuals aged ≥18 years, time since GH discontinuation positively correlated with BIA fat percentage; Spearman's rank correlation coefficient 0.525, p=0.054. BIA FMI; Spearman's rank correlation coefficient 0.534, p=0.049. There were no significant correlations between time since GH treatment in the above parameters in individuals aged <18 years.

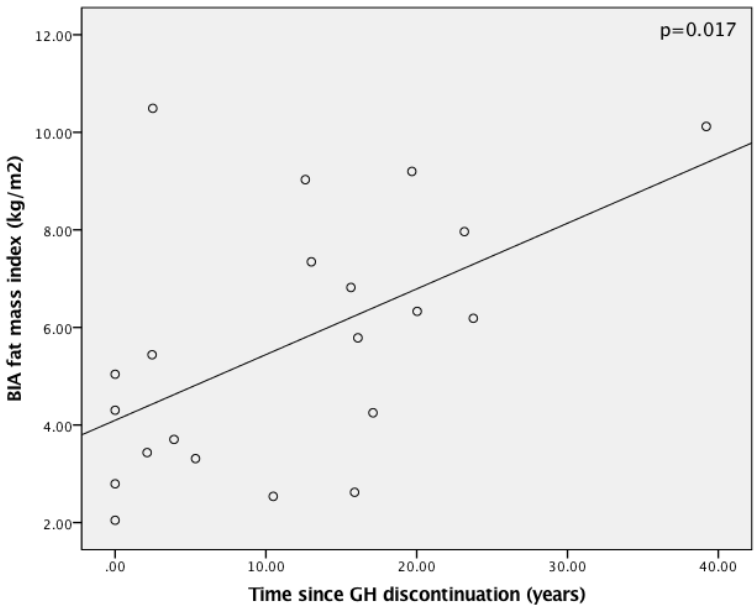


Figure 7.3 Scatter plot of BIA fat mass index against time since GH discontinuation. Line of best fit indicated.

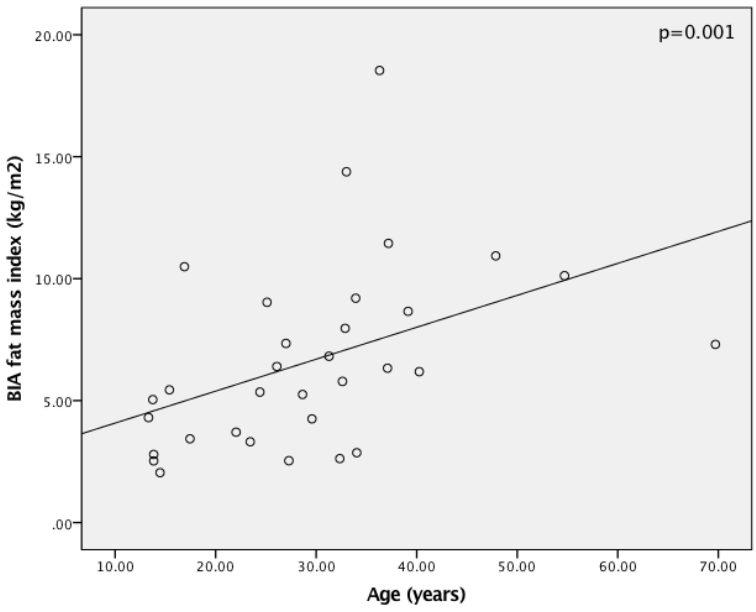


Figure 7.4 Scatter plot of BIA fat mass index against age. Line of best fit indicated.

7.3.3 Lean mass in SRS

Fat-free mass index (FFMI) was calculated from BIA and DXA. Lean mass index (LMI) was also calculated from DXA. Overall FFMI was 14.73 kg/m^2 by BIA and 8.03 kg/m^2 by DXA. LMI by DXA was 12.45 kg/m^2. In individuals aged ≥18 years, FFMI was 16.27 kg/m^{2}by BIA and 13.13 kg/m^2 by DXA. LMI was 9.33 kg/m^2 by DXA. In individuals <18 years FFMI was 14.36 kg/m^{2}by BIA and 13.02 kg/m^2 by DXA. LMI was 12.45 kg/m^2 by DXA.

Median BIA FFMI was 16.27 kg/m^2 in aged ≥18 years and 14.65 kg/m^2 in individuals aged <18 years (p=0.033). Median DXA fat-free mass index in individuals aged ≥18 years and in individuals aged <18 years were 13.13 and 13.02 respectively (p=0.837). LMI in individuals aged ≥18 years and in individuals aged <18 years old were 12.38 and 12.45 respectively (p=0.837) (Table 7.3).

Overall BMI SDS correlated with FFMI from BIA; Spearman's rank correlation coefficient 0.801, p<0.001. There were weaker correlations with FFMI from DXA and LMI from DXA which did not reach significance; Spearman's rank correlation coefficient 0.365, p=0.094 and Spearman's rank correlation coefficient 0.365, p=0.094 respectively. In individuals aged ≥18 years BMI SDS correlated with FFMI from BIA; Spearman's rank correlation coefficient 0.813, p<0.001. Similarly, correlations with FFMI from DXA and LMI from DXA did not reach significance; Spearman's rank correlation coefficient 0.399, p=0.101 in both cases.

Serum creatinine was measured in the study and is a biochemical marker of muscle mass. Overall, the median creatinine level was 56.0 μmol/L. The University Hospital Southampton laboratory reference ranges were used to determine low creatinine levels, which were present in 54.5%. The

normal ranges are age-specific from birth to 11 years, then age- and sex-specific from 11 to 18 years, and from 18 years onwards there is a single normal range for each sex. In individuals aged ≥18 years the median creatinine level was 67.0 μmol/L and 68.0% (17/25) had a low creatinine level according to the laboratory reference range. Individuals aged <18 years had a median creatinine level of 50.5 μmol/L and 12.5% had a low creatinine according to the laboratory reference range.

No significant correlation was found between BMI SDS and creatinine level in individuals ≥18 years; Spearman's rank correlation -0.279, p=0.177, or individuals <18 years; Spearman's rank correlation 0.252, p=0.548.

7.3.4 Lean mass and the effects of GH treatment in SRS

FFMI and LMI were similar in GH-treated than GH-untreated individuals overall (Table 7.1) and in individuals aged ≥18 years only (Table 7.2).

In the overall cohort, individuals aged ≥18 years only and individuals aged <18 years, there were no significant correlations between time since GH discontinuation and BIA FFMI, DXA FFMI or DXA LMI.

GH-untreated and GH-treated individuals aged ≥18 years had median creatinine levels of 59.0 (IQR 45.5 to 73.3, n=10) and 68.0 (53.0 to 72.0, n=15) respectively (p=0.397) and there was no difference in the prevalence of low creatinine levels; 70.0% and 66.7% respectively (p=1.000).

7.3.5 Muscle function in SRS

Muscle function was assessed by measuring hand grip strength and the maximum grip strength was converted into age- and sex-dependent SDS as described in section 3.7.8.

Overall median grip strength SDS was -1.76 (IQR -2.47 to -1.25). Median grip strength SDS in individuals aged ≥18 years was -2.12 (IQR -2.90 to -1.57) (n=22) and -1.31 (IQR -1.50 to 0.18) in individuals <18 years old (n=8) (p=0.006).

Grip strength positively correlated with DXA FFMI and DXA LMI in the overall cohort and individuals aged ≥18 years only. In individuals aged ≥18 years grip strength also correlated with BIA FFMI (Table 7.4).

Table 7.4 Correlations between grip strength and FFMI, LMI and serum creatinine levels. P values in bold denote significance.

	Overall cohort		Individuals aged ≥ 18 years		Individuals aged <18 years	
	Spearman's rank correlation coefficient	P value	Spearman's rank correlation coefficient	P value	Spearman's rank correlation coefficient	P value
Grip strength SDS and BIA FFMI	0.251	0.131	0.582	**0.004**	0.434	0.283
Grip strength SDS and DXA FFMI	0.604	**0.006**	0.694	**0.004**	0.400	0.600
Grip strength SDS and DXA LMI	0.604	**0.006**	0.694	**0.004**	0.400	0.600
Grip strength SDS and creatinine	0.017	0.929	0.311	0.159	0.301	0.468

7.3.6 Muscle function and the effects of GH treatment in SRS

Median grip strength SDS in GH-untreated and GH-treated individuals were -2.13 (IQR -2.63 to -0.89) (n=9) and -1.73 (IQR -2.50 to -1.31) (n=21) respectively (p=0.928).

In the overall cohort, grip strength SDS negatively correlated with time since discontinuation of GH treatment; Spearman's rank correlation coefficient -0.524, p=0.018 (see Figure 7.5). However, this relationship was not significant in those ≥18 years only; Spearman's rank correlation coefficient -0.173, p=0.571 or those <18 years 0.461, p=0.297. There was a suggestion that grip strength SDS also negatively correlated with age; Spearman's rank correlation coefficient -0.308, p=0.097 (see Figure 7.6).

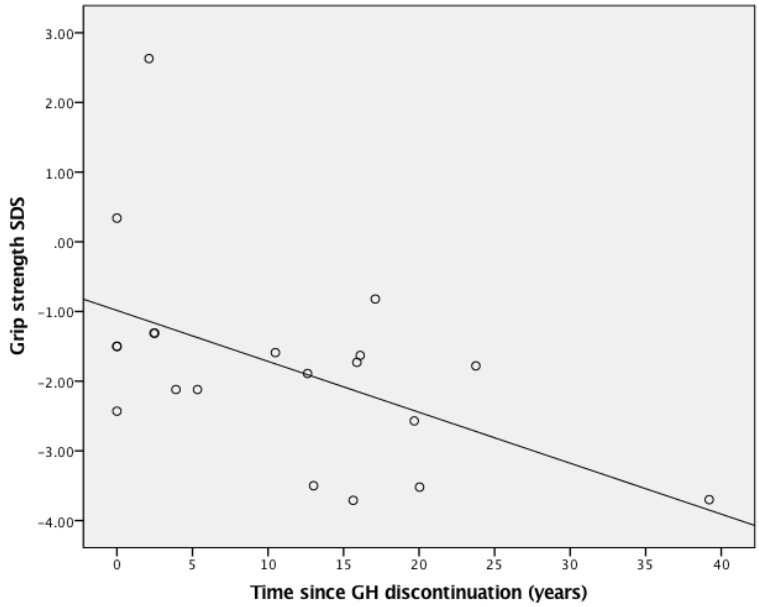


Figure 7.5 Scatter plot of grip strength SDS against time since GH discontinuation. Line of best fit displayed.

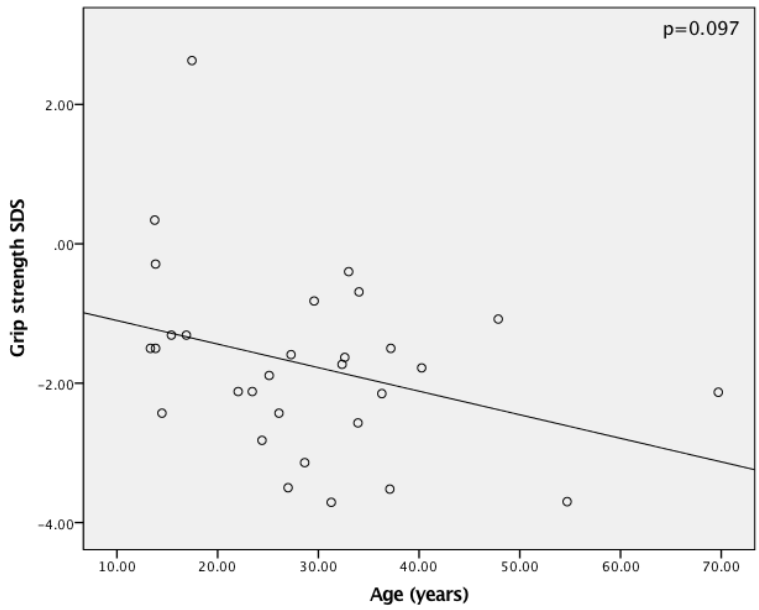


Figure 7.6 Scatter plot of grip strength SDS against age. Line of best fit displayed.

7.3.7 Bone mineral density in SRS

Bone mineral density (BMD) was measured by DXA scan (see methods section 3.7.7). Figure 7.7 shows an example of the output of DXA bone analysis.

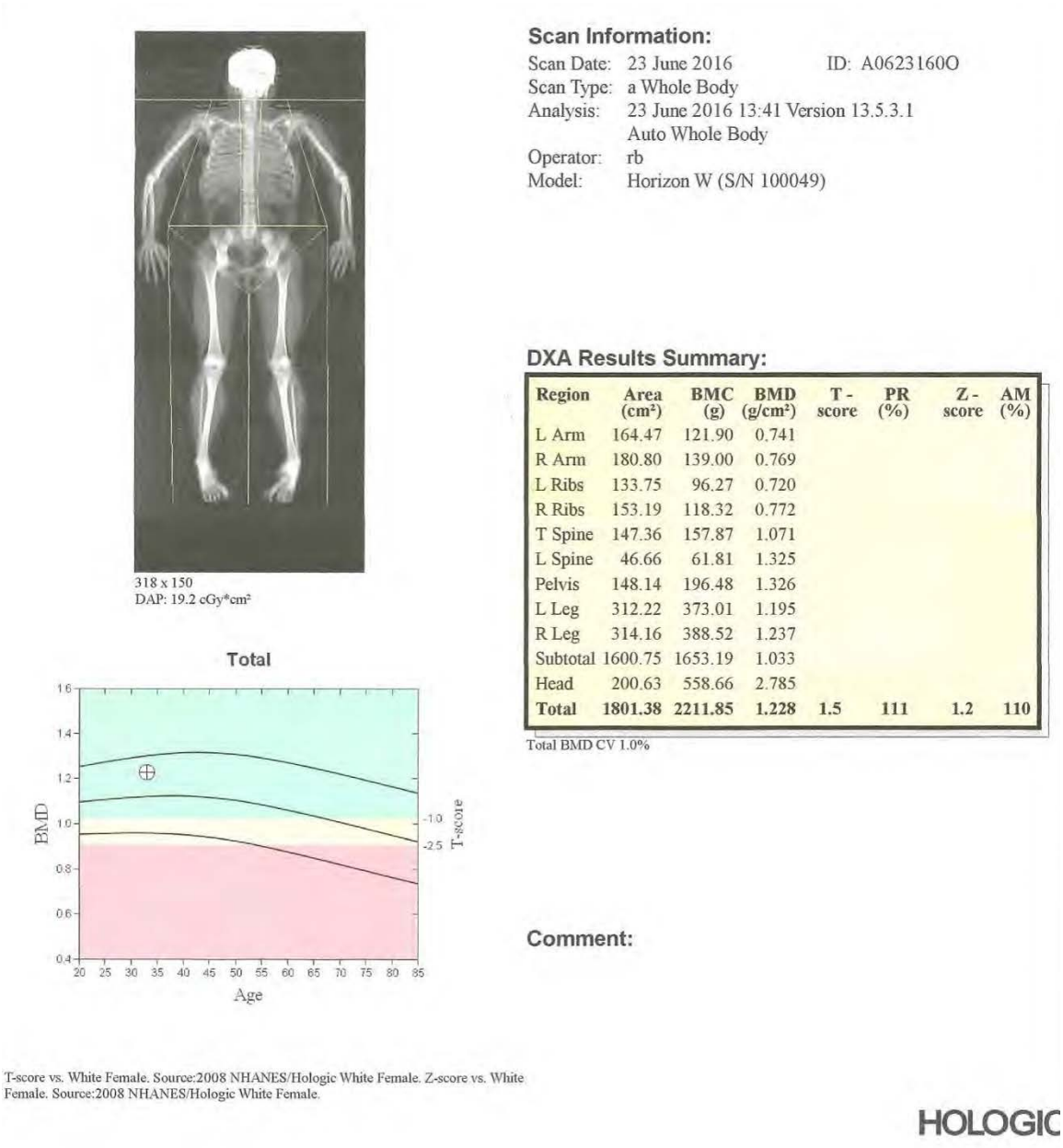


Figure 7.7 An example of the Hologic DXA bone analysis output. This result was obtained from a STAARS participant (Case ID 27).

The BMD results for the cohort are summarised in

Table 7.5. Spine BMAD Z-scores were higher in individuals aged ≥18 years than those <18 years; median values 0.70 vs -0.80 (p=0.04). There was a suggestion that whole body total BMD Z-scores were also higher in individuals aged ≥18 years than those <18 years; -0.60 vs -1.75 (p=0.081). Total hip BMD Z-scores were higher in individuals aged ≥18 years than those <18 years but again, did not reach statistical significance; -0.60 vs -1.30 (p=0.087).

Table 7.5 DXA bone results separated into age subgroups. Bone mineral density (BMD) in g/cm^2, bone mineral apparent density (BMAD) in g/cm^3. Results show median and interquartile ranges. *n=3 and full range given. ^n=18.

	Individuals aged ≥18 years	Individuals aged <18 years	P value
	n=19	n=4	
DXA whole body BMD	1.083 (1.037 to 1.137)^	0.931 (0.876 to 0.980)	**0.002**
DXA whole body BMD Z-score	-0.60 (-1.55 to -0.30)^	-1.75 (-1.80 to -1.18)	0.081
DXA whole body BMD T-score	-0.65 (-1.65 to -0.30)^	n/a	
DXA subtotal BMD	0.928 (0.855 to 1.007)^	0.852 (0.760 to 0.880)	0.098
DXA subtotal BMD Z-score	n/a	-1.50 (-2.10 to -1.05)	
DXA total hip BMD	0.880 (0.798 to 0.938)	0.841 (0.761 to 0.852)*	0.408
DXA total hip BMD Z-score	-0.60 (-1.10 to 0.00)	-1.30 (-1.70 to -1.20)*	0.087
Spine BMAD	0.272 (0.242 to 0.286)	0.216 (0.196 to 0.262) *	**0.040**
Spine BMAD Z-score	0.70 (-0.10 to 1.30)	-0.80 (-1.40 to 0.40)*	**0.040**

The WHO criterion for a diagnosis of osteoporosis in adults is a T-score ≤-2.50 at the femoral neck and T-scores -1.0 to -2.5 are consistent with osteopenia (305). The diagnosis of osteoporosis in a child or adolescent requires the presence of both a clinically significant fracture history and low aBMD or BMC for chronological age (306). There was one individual with a T-score ≤-2.50 who was in the group aged ≥18 years. The T-score was -3.10. Another individual had been diagnosed with osteopenia prior to the study. In individuals aged ≥18 years, 33.3% had a total BMD T-score of -1.0 to -2.5, consistent with osteopenia.

In the cohort overall, BMI SDS positively correlated with whole body BMD Z-score; Spearman rank correlation coefficient 0.519, p=0.013, total hip BMD Z-score; Spearman rank correlation coefficient 0.592, p=0.004 but did not correlate significantly with spine BMAD Z-score; Spearman rank correlation coefficient 0.340, p=0.121. There was a tendency for underweight weight status to be associated with osteopenia but this did not reach statistical significance (p=0.083). However, when the osteopenic individuals were compared to those who were not, there was a significant difference in BMI SDS; p=0.010.

In individuals aged ≥18 years, BMI SDS positively correlated with total BMD Z-score; Spearman rank correlation coefficient 0.579, p=0.012 (see Figure 7.8), total BMD T-score; Spearman's rank correlation coefficient 0.636, p= 0.005 (see Figure 7.9), total hip BMD Z-score; Spearman rank correlation coefficient 0.521, p=0.022 (see Figure 7.10) but did not correlate significantly with spine BMAD Z-score; Spearman rank correlation coefficient 0.284, p=0.238.

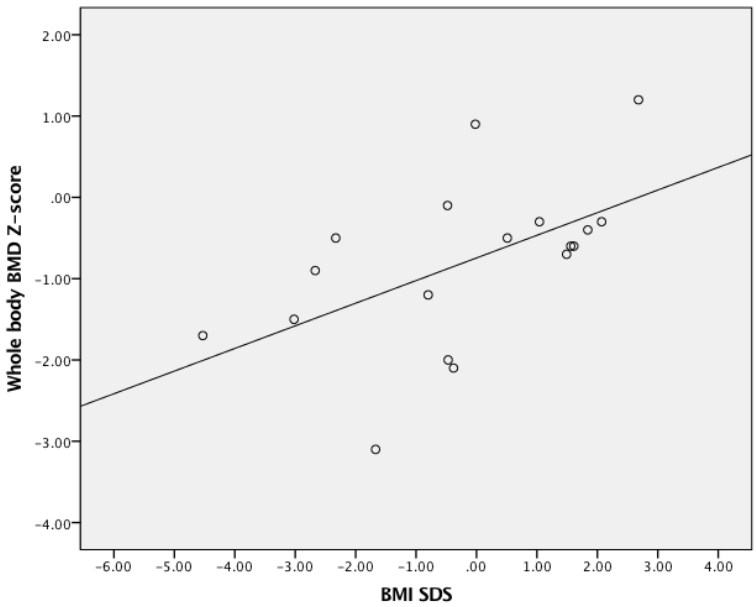


Figure 7.8 Scatter plot of whole body BMD Z-score against BMI SDS. Line of best fit indicated.

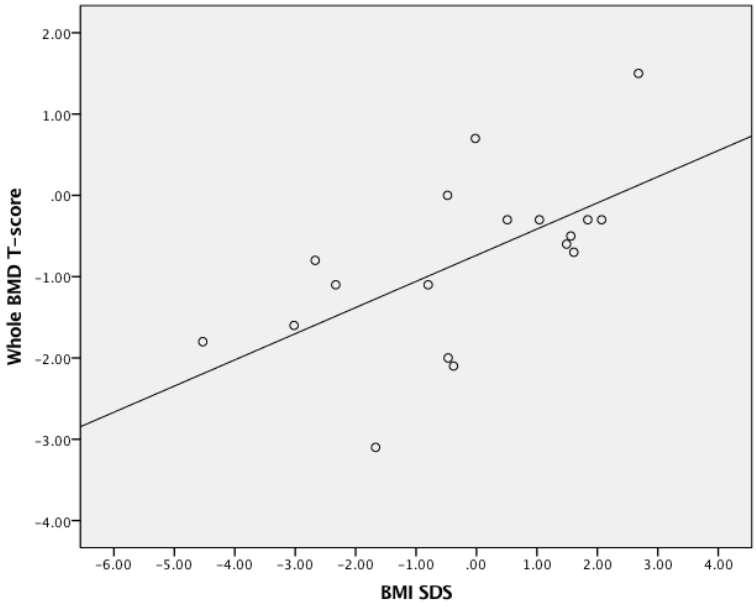


Figure 7.9 Scatter plot of whole body BMD T-score against BMI SDS. Line of best fit indicated.

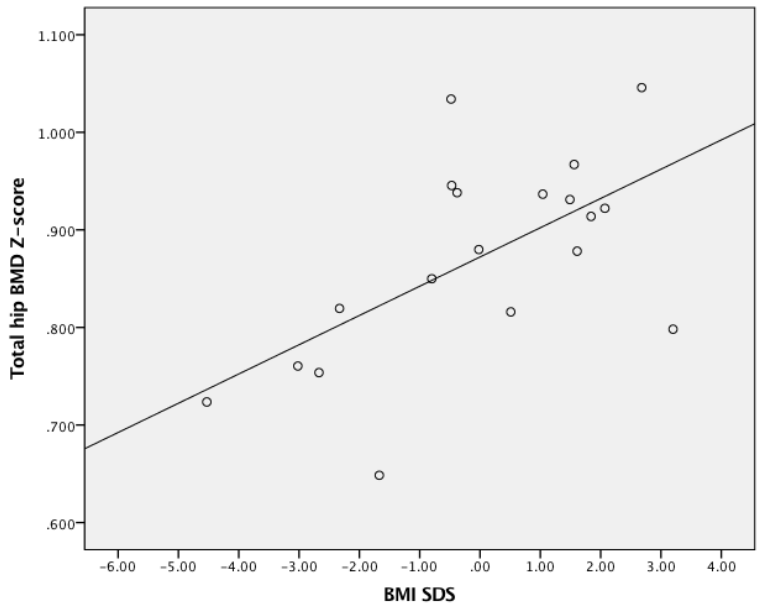


Figure 7.10 Scatter plot of total hip BMD Z-score against BMI SDS. Line of best fit indicated.

7.3.8 Bone mineral density and the effects of GH treatment in SRS

Table 7.6 shows the overall comparison of GH treatment groups. Median spine BMAD was greater in GH-untreated than GH-treated individuals (p=0.025) and there was a suggestion of greater spine BMAD Z-scores; 0.90 and 0.60 respectively (p=0.071). There were no significant differences in whole body BMD Z-score or total hip BMD Z-score between GH-untreated individuals and GH-treated individuals.

Table 7.7 shows the comparison of GH treatment groups only including individuals aged ≥18 years. There were no significant differences in BMD or BMAD. There was no association between osteopenia and previous GH treatment (p=0.563).

There were no significant correlations between time since GH discontinuation and total BMD Z-score, total BMD T-score, total hip BMD Z-score, or spine BMAD Z-score in the overall cohort or in individuals aged ≥18 years only. In those aged <18 years, the duration of time since GH discontinuation correlated strongly with total hip BMD Z-score (Spearman's rank correlation coefficient 1.000, p=0.01).

Table 7.6 DXA bone results of the UK STAARS cohort and separated by GH treatment group. Bone mineral density (BMD) in g/cm^2, bone mineral apparent density (BMAD) in g/cm^3. Results show median and interquartile ranges except where indicated: ▲n=4 *n=8 △ n=10 ^n=13 ▫n=18.

	All	GH-untreated individuals	GH-treated individuals	P value
Number	22	9	14	
DXA whole body BMD	1.064 (0.980 to 1.114)	1.086 (1.041 to 1.146)	1.040 (0.941 to 1.114)	0.165
DXA whole body BMD Z-score	-0.80 (-1.73 to -0.38)	-0.55 (-1.13 to -0.33)*	-1.25 (-1.80 to -0.45)	0.238
DXA whole body BMD T-score	-0.65 (-1.65 to -0.30)▫	-0.75 (-1.10 to -0.30)*	-0.55 (-1.88 to -0.23)△	0.897
DXA subtotal BMD	0.879 (0.850 to 0.996)	0.936 (0.848 to 1.012)*	0.870 (0.852 to 0.920)	0.616
DXA subtotal BMD Z-score	-1.50 (-2.10 to -1.05)▲		-1.50 (-2.10 to -1.05)▲	
DXA total hip BMD	0.865 (0.789 to 0.937)	0.878 (0.809 to 0.934)	0.852 (0.761 to 0.937)^	0.744
DXA total hip BMD Z-score	-0.70 (-1.35 to -0.08)	-0.60 (-1.10 to -0.05)	-1.00 (-1.65 to -0.15)^	0.292
Spine BMAD	0.269 (0.235 to 0.285)	0.284 (0.261 to 0.296)	0.262 (0.217 to 0.273)^	**0.025**
Spine BMAD Z-score	0.60 (-0.38 to 1.08)	0.90 (0.15 to 1.50)	0.60 (-0.75 to 0.80)^	0.071

Table 7.7 DXA bone results of the individuals aged ≥ 18 years in the UK STAARS cohort and stratified by GH treatment group. Bone mineral density (BMD) in g/cm^2, bone mineral apparent density (BMAD) in g/cm^3. Results show median and interquartile ranges except where indicated. ▲n=18 *n=8.

	Individuals aged ≥18 years	GH-untreated individuals aged ≥18 years	GH-treated individuals aged ≥18 years	P value
Number	19	9	10	
DXA whole body BMD	1.083 (1.037 to 1.137)▲	1.085 (1.041 to 1.146)*	1.080 (1.025 to 1.137)	0.633
DXA whole body BMD Z-score	-0.60 (-1.55 to -0.30)▲	-0.55 (-1.13 to -0.33)*	-0.65 (-1.80 to -0.25)	0.573
DXA whole body BMD T-score	-0.65 (-1.65 to -0.30)▲	-0.75 (-1.10 to -0.30)*	-0.55 (-1.88 to -0.23)	0.897
DXA subtotal BMD	0.928 (0.855 to 1.007)▲	0.936 (0.848 to 1.012)*	0.899 (0.860 to 1.006)	0.965
DXA total hip BMD	0.880 (0.798 to 0.938)	0.878 (0.809 to 0.934)	0.905 (0.751 to 0.945)	1.000
DXA total hip BMD Z-score	-0.60 (-1.10 to 0.00)	-0.60 (-1.10 to -0.05)	-0.60 (-1.70 to 0.08)	0.720
Spine BMAD	0.272 (0.242 to 0.286)	0.284 (0.261 to 0.296)	0.268 (0.230 to 0.274)	0.079
Spine BMAD Z-score	0.70 (-0.10 to 1.30)	0.90 (0.15 to 1.50)	0.60 (-0.40 to 0.93)	0.182

7.4 Metabolic findings

7.4.1 Biochemical results in SRS overall

Metabolic results from the STAARS UK cohort, stratified by age group, are shown in Table 7.10 shows biochemistry and metabolic results categorised as high or low values according to clinical guidance so that the percentage of abnormal cases can be visualised for any one test (triglycerides, total cholesterol, HDL cholesterol, LDL cholesterol, metabolic syndrome, waist circumference, blood pressure, BP) or laboratory reference range (ALT, AST, GGT, IGF1, insulin) or both (glucose). An elevated fasting glucose (≥6.1 mmol/L) was found in 20.0% individuals overall. All cases with an elevated fasting glucose were aged ≥18 years; the prevalence was 25.0% including only those aged ≥18 years. Blood glucose concentration positively correlated with age; Spearman rank correlation coefficient 0.396, p=0.03 (see Figure 7.11).

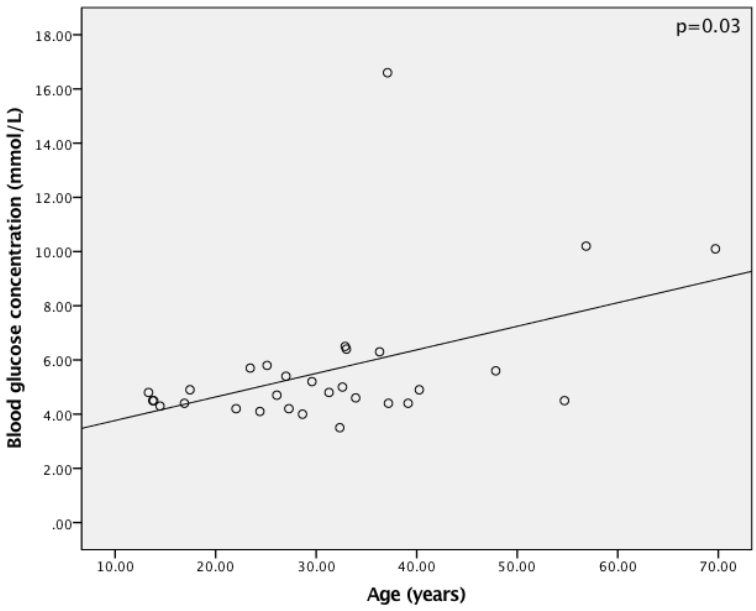


Figure 7.11 Scatter plot of blood glucose concentration against age. Line of best fit indicated.

7.4.1.1 Biochemical results in individuals with SRS ≥18 years

Two individuals were known to have type 2 diabetes mellitus; one male aged 69.71 years who had a fasting blood glucose concentration of 10.10 mmol/L and HbA1c of 42 mmol/mol (case ID 25) and the other, a female aged 56.85 years who had a fasting blood glucose of 10.2 mmol/L and HbA1c of 53 mmol/mol (case ID 29). One female reported impaired glucose tolerance and had a fasting blood glucose of 6.3 mmol/L and a HbA1c of 40 mmol/mol (case ID 5). As a result of the

research study investigations, two individuals were found to have impaired fasting glycaemia. One male aged 32.88 years had a fasting blood glucose of 6.5 mmol/L and an HbA1c of 35 mmol/mol (case ID 6). The other was a female aged 33.01 years who had a fasting blood glucose level of 6.4 mmol/L and a HbA1c of 30 mmol/mol (case ID 27). One male aged 37.09 years was diagnosed with type 2 diabetes as a result of the study investigations; the fasting blood glucose concentration was 16.6 mmol/L and HbA1c was 102 mmol/mol (case ID 10).

Raised GGT was observed in three females aged ≥18 years. The first had H19/IGF2 LOM, was aged 36.30 years, and had a BMI SDS of 3.2. The raised GGT was associated with an elevated ALT but not AST and diagnoses of non-alcoholic hepatitis and treated hypothyroidism were reported. TSH level was normal (case ID; the same individual is also described above with impaired glucose tolerance). The second individual was aged 33.93 years, had matUPD7 and a BMI SDS of 0.51. Neither ALT nor AST were elevated in this case. There was a reported diagnosis of treated hypothyroidism and TSH level was normal (case ID 23). The third individual was aged 33.01 years, had H19/IGF2 LOM and a BMI SDS of 2.68, ALT and AST not elevated. There was reported history of anorexia and bulimia (case ID 27).

In the cohort overall as well as exclusively those aged ≥18 years, there was a positive correlation between body fat percentage from SFTs and HOMA-IR (see Table 7.8).

Table 7.8 Correlations between body fat percentage, FMI and FFMI and blood glucose concentrations, HbA1c and HOMA-IR. P values in bold denote significance.

	Overall cohort		Individuals aged ≥ 18 years		Individuals aged <18 years	
	Spearman's rank correlation coefficient	P value	Spearman's rank correlation coefficient	P value	Spearman's rank correlation coefficient	P value
Body fat percentage from SFTs and glucose	0.183	0.360	0.145	0.530	-0.029	0.957
Body fat percentage from SFTs and HbA1c	0.175	0.354	0.222	0.321	-0.419	0.301
Body fat percentage from SFTs and HOMA-IR	0.497	**0.016**	0.615	**0.007**	0.600	0.285
BIA fat percentage and glucose	0.403	**0.030**	0.431	**0.040**	0.203	0.700
BIA fat percentage and HbA1c	0.294	0.102	0.388	0.061	-0.084	0.844
BIA fat percentage and HOMA-IR	0.305	0.138	0.337	0.146	0.7	0.188
BIA FMI and glucose	0.393	**0.035**	0.414	**0.049**	0.116	0.827
BIA FMI and HbA1c	0.156	0.393	0.176	0.410	-0.024	0.955
BIA FMI and HOMA-IR	0.455	**0.022**	0.534	**0.015**	0.6	0.285
BIA FFMI and glucose	0.012	0.952	0.005	0.982	0.058	0.913
BIA FFMI and HbA1c	-0.292	0.105	-0.357	0.087	-0.192	0.649
BIA FFMI and HOMA-IR	0.364	0.074	0.483	**0.031**	-0.8	0.104

7.4.1.2 Biochemical results in individuals with SRS <18 years

Three individuals aged <18 years had results of note: the first had an elevated waist circumference and BMI SDS of 1.53 but normal levels of cholesterol, triglycerides, ALT, AST and

GGT (case ID 3). The second had isolated hypercholesterolaemia with total cholesterol of 5.4 mmol/L and raised LDL cholesterol of 3.2 mmol/L but a BMI SDS of -0.53 and SFT body fat percentage of 15.1%. In this case the ALT was normal and AST and GGT were not available (case ID 41). The third individual had a raised AST of 48 iu/L but normal ALT, GGT, triglycerides and glucose levels. The same individual had a BMI SDS of -1.33 and SFT body fat percentage of 16.7% and did not have an elevated waist circumference (case ID 37).

7.4.1.3 Comparison of biochemical results in SRS age subgroups

Comparing individuals aged ≥18 years to those aged <18 years, gamma glutamyl transferase (GGT) was significantly higher and IGF1 levels were significantly lower. However, there was no significant difference in IGF1 SDS values. High IGF1 levels were less prevalent in individuals aged ≥18 years (Table 7.10).

7.4.1.4 Biochemical results and the effects of GH treatment in SRS

Comparisons between GH treatment groups were made only including individuals aged ≥18 years (Table 7.1) because all individuals aged <18 years were treated with GH. Triglyceride levels were significantly higher in GH-untreated individuals.

Table 7.9 Biochemistry and metabolic results separated by age subgroup. Total cholesterol, HDL cholesterol, non-HDL cholesterol, LDL cholesterol, triglycerides, creatinine and glucose in mmol/L. Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma glutamyl transferase (GGT) in (international) units/L. HbA1c in mmol/mol. Insulin in mU/L. C-peptide in pmol/L. IGF1 in nmol/L. *n=24. ^n=23. ▫n=20. ‣n=22. ▽n=15. △n=7. △n=7. ▪n=6. ▲n=5.

	Individuals ≥18 years	Individuals aged <18 years	P value
Number	25	8	
Total cholesterol	5.00 (4.30 to 5.75)	4.30 (4.20 to 4.90)△	0.224
HDL cholesterol	1.53 (1.42 to 1.83)*	1.65 (1.54 to 1.69)△	0.341
non-HDL cholesterol	3.15 (2.66 to 3.82)▽	3.14 (2.50 to 3.37)▲	0.553
LDL cholesterol	2.55 (2.05 to 3.47)‣	2.43 (2.23 to 2.85)△	0.469
cholesterol/HDL ratio	3.16 (2.48 to 3.98)*	2.85 (2.58 to 2.89)^	0.234
Triglycerides	1.05 (0.80 to 1.58)*	0.80 (0.70 to 1.10)△	0.341
ALT	23.0 (15.5 to 35.0)	18.5 (15.5 to 35.5)	0.757
AST	25.5 (22.0 to 31.8)*	24.5 (21.3 to 30.8)▪	0.781
GGT	24.0 (18.0 to 38.3)*	12.5 (12.0 to 14.0)▪	**<0.001**
Glucose	4.95 (4.40 to 6.18)*	4.50 (4.38 to 4.83)▪	0.230
HbA1c	32.0 (30.0 to 38.0)	32.5 (30.3 to 33.8)	0.821
Insulin	3.60 (2.90 to 8.10)^	4.50 (3.45 to 7.33)▪	0.773
C-peptide	490.0 (330.0 to 748.0)^	447.0 (395.5 to 629.3)▪	0.813
HOMA-IR	1.05 (0.55 to 2.29)▫	0.958 (0.790 to 1.76)▲	0.869
QUICKI	0.383 (0.338 to 0.427)▫	0.386 (0.354 to 0.400)▲	0.869
IGF1	22.2 (18.2 to 29.9)*	79.2 (27.3 to 146.6)△	**0.004**
IGF1 SDS	0.73 (-0.43 to 1.40)*	2.31 ('1.12 to 5.07)^	0.317

Table 7.10 Biochemistry and metabolic results of the overall STAARS UK cohort and age subgroups categorised as high or low values. P values shown of comparison between individuals aged ≥18 years and individuals aged <18 years.

	All	Individuals aged >=18 years	Individuals aged <18 years	P value
High triglycerides	**16.1% (5/31)**	**20.8% (5/24)**	**0% (0/7)**	**0.312**
High total cholesterol	**43.8% (14/32)**	**52.0% (13/25)**	**14.3% (1/7)**	**0.104**
Low HDL chol	**0% (0/31)**	**0% (0/24)**	**0% (0/7)**	
High LDL chol	**33.3% (10/30)**	**39.1% (9/23)**	**14.3% (1/7)**	**0.372**
High ALT	**12.1% (4/33)**	**16.0% (4/25)**	**0% (0/8)**	**0.550**
High AST	**3.3% (1/30)**	**0% (0/24)**	**16.7% (1/6)**	**0.200**
High GGT	**10.0% (3/30)**	**12.5% (3/24)**	**0% (0/6)**	**0.592**
High glucose	**20.0% (6/30)**	**25.0% (6/24)**	**0% (0/6)**	**0.302**
High insulin	**3.4% (1/29)**	**4.3% (1/23)**	**0% (0/6)**	**1.000**
High IGF1	**12.9% (4/31)**	**4.2% (1/24)**	**42.9% (3/7)**	**0.028**
Metabolic syndrome	**15.4% (4/26)**	**18.2 (4/22)**	**0% (0/4)**	**0.588**
High waist circumference	**30.3% (10/33)**	**36.0% (9/25)**	**12.5% (1/8)**	**0.382**
High BP	**27.6% (8/29)**	**33.3% (8/24)**	**0% (0/5)**	**0.283**

Table 7.11 Biochemistry and metabolic results of individuals aged ≥18 years separated by GH treatment group. Total cholesterol, HDL cholesterol, non-HDL cholesterol, LDL cholesterol, triglycerides, creatinine and glucose in mmol/L. Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma glutamyl transferase (GGT) in (international) units/L. HbA1c in mmol/mol. Insulin in mU/L. C-peptide in pmol/L. ^n=7 ▲n=8 *n=9 ▪n=12 ▫n=13 ▽n=14.

	GH-untreated individuals	GH-treated individuals	P value
Number	10	15	
Total cholesterol	4.90 (4.25 to 5.88)	5.00 (4.50 to 5.80)	0.807
HDL cholesterol	1.53 (1.46 to 1.75)	1.53 (1.40 to 1.99)▽	0.886
non-HDL cholesterol	3.15 (2.70 to 3.82)^	3.14 (2.51 to 3.81)▲	0.779
LDL cholesterol	2.51 (2.00 to 3.10)*	2.72 (2.01 to 3.79)▫	0.471
Cholesterol/HDL cholesterol ratio	3.49 (2.54 to 3.86)	3.04 (2.44 to 4.23)▽	0.666
Triglycerides	1.50 (1.00 to 2.15)*	0.90 (0.70 to 1.20)	**0.041**
ALT	21.0 (10.5 to 39.8)	25.0 (18.0 to 33.0)	0.567
AST	25.0 (20.8 to 27.5)	25.5 (22.0 to 34.0)▽	0.546
GGT	23.5 (17.8 to 41.5)	24.0 (18.0 to 35.3)▽	0.709
Creatinine	59.0 (45.5 to 73.3)	68.0 (53.0 to 72.0)	0.397
Glucose	4.7 (4.3 to 6.4)*	5.0 (4.5 to 5.8)	0.682
HbA1c	32.5 (29.5 to 39.3)	32.0 (30.0 to 36.0)	0.849
Insulin	7.7 (3.5 to 16.7)*	3.15 (2.63 to 6.83)▽	0.083
C-peptide	583.0 (407.0 to 1251.5)*	446.5 (321.3 to 736.0)▽	0.277
HOMA-IR	1.55 (0.34 - 5.65)▲	0.731 (0.473 to 1.74)▪	0.270
QUICKI	0.36 (0.30 -0.47)▲	0.405 (0.351 to 0.438)▪	0.270
IGF1	22.2 (18.0 to 29.7)	21.5 (18.1 to 35.0)▽	0.886
IGF1 SDS	0.87 (-0.28 to 1.49)	0.57 (-0.63 to 1.41)▽	0.666

7.4.2 Metabolic syndrome in SRS

Using the Alberti criteria for the diagnosis of metabolic syndrome (as described in section 3.6.2), the overall prevalence in the STAARS UK cohort was 15.4% (4/26). All individuals diagnosed with metabolic syndrome were aged ≥18 years and the prevalence in that subgroup was 18.2% (4/22). The difference in the prevalence of metabolic syndrome between the age subgroups was not significant (p=0.588) (table 6.9).

Overall prevalence of hypertension was 27.6% (8/29) and in individuals aged ≥18 years 33.3% (8/24). Although there were no individuals aged <18 years who met the Overall, metabolic syndrome was present in 33.3% (3/9) of GH-untreated individuals and 5.9% (1/17) in GH-treated individuals (p=0.104). In individuals aged ≥18 years, metabolic syndrome was present in 33.3% (3/9) in GH-untreated individuals and 7/7% (1/13) in GH-treated individuals (p=0.264). The International Diabetes Federation estimates the prevalence of metabolic syndrome in adults to be 25% worldwide (307).

Comparing the groups with and without metabolic syndrome aged ≥18 years, there were significant differences in BIA fat percentage; median 40.7% and 31.1% respectively (p=0.04) and BIA fat mass index; median 12.7 kg/m^2 and 6.8 kg/ m^2 respectively (p=0.04).

7.4.3 Hypertension in SRS

Overall prevalence of hypertension was 27.6% (8/29) and in individuals aged ≥18 years 33.3% (8/24). Although there were no individuals aged <18 years who met the criteria for hypertension, a significant difference between the age subgroups was not demonstrated (p=0.283).

Overall, hypertension was present in 30.0% (3/10) of GH-untreated individuals and 26.3% (5/19) of GH-treated individuals (p=1.000). In individuals aged ≥18 years, hypertension was present in 30.0% (3/10) of GH-untreated individuals and 35.7% (5/14) of GH-treated individuals (p=0.264).

7.5 Discussion

7.5.1 Body composition, metabolic findings and cardiovascular risk factors in adults with SRS

7.5.1.1 Body composition

This study demonstrated that adults with SRS (i.e. aged ≥ 18 years) were underweight in 20%, ideal weight in 44%, overweight in 24%, obese class I in 8%, and obese class II in 4%. Obesity of any class was only seen in the GH untreated group. The prevalence of elevated waist

circumference was 36%. The prevalence of obesity seen here was lower than 27% reported in adults in the UK in 2015 and the prevalence of overweight seen here is lower than 41% reported in adult males and 31% reported in adult females. Elevated waist circumference was slightly lower than the 2015 UK prevalence in adults which found 35% of men had a 'very high waist circumference' (>102 cm) and 47% of women (>88 cm) (308). However, the UK prevalence included individuals aged ≥16 years old and the definitions of waist circumference were higher than those used in this study. This would suggest that the UK prevalence would be greater if lower limits were applied and therefore the results seen here would still reflect a lower than average prevalence of elevated waist circumference. The weight distribution in this study does not appear to reflect excessive overweight or obesity. Underweight body status was notably present and at similar levels to those of 23.4% in men and 24.0% in women in South Asia, which had the highest prevalence of underweight in a study in 2014 (309). In this study, the mean waist-to-hip ratio in males and females were 0.928 and 0.830 respectively. These values are at the limits of the ideal waist-to-hip ratios of <0.95 in males and <0.80 females (249) suggesting that there is some but not marked central adiposity in SRS.

There were conflicting results from the correlations with BMI SDS: there was a stronger correlation between BMI SDS and DXA body fat percentage/fat mass than between BMI SDS and DXA FFMI/LMI. However, there was a stronger correlation between BMI SDS and BIA FFMI than between BMI SDS and BIA body fat percentage/FMI. These results are difficult to interpret, however the DXA results may be more reliable as DXA remains the gold standard in body composition analysis. DXA body fat percentage and fat mass index correlated more strongly with BMI SDS than fat-free mass which may suggest that adiposity is more significant in SRS than sarcopenia.

Creatinine levels were low in 70.0% of those aged ≥18 years which suggests there was low muscle mass which might relate to smaller overall body size or low muscle mass for height. Grip strength was generally low and positively correlated with DXA lean mass measures (FFMI and LMI) which would reflect muscle function correlating with muscle mass. However, grip strength did not correlate with BMI SDS or serum creatinine level, suggesting that these were poor markers of muscle mass or that muscle mass did not directly correlate with muscle function. BMI SDS is likely to poorly reflect muscle mass as there is no differentiation in for muscle and fat in its calculation. Creatinine levels were frequently low, however the utility of the absolute value (as utilised in the correlation analysis) is affected by renal function and hydration therefore these factors may have affected the observed correlation.

In this study, the median total body BMD observed was 1.083 g/m^2 and the median whole body BMD Z-score was -0.60. In individuals aged ≥18 the prevalence of osteopenia was 33.3%. BMI

positively correlated with BMD and T-score and BMD Z-score but not spine BMAD which might reflect that weight as a load-bearing force affects the spine less than more dependent body parts. However, individuals with osteopenia had significantly lower BMI SDS than those without as might be expected. In individuals ≥18 years, median total body fat was 24.25% (SFTs), 31.75% (BIA), and 44.45% (DXA). BIA FMI was 6.61 kg/m^2 and DXA FMI was 9.33 kg/m^2. BIA FFMI was 16.27 kg/m^{2}and DXA FFMI was 13.13 kg/m^2. DXA LMI was 9.33 kg/m^2. These results are supported by a small study of seven individuals with molecularly confirmed SRS which reported: fat mass percentage 38.2% ± 10.2 (range 26-55.7%), fat mass index 4-17.4 kg/m^2. The same study found similar waist-to-hip ratios of 0.87-0.90 in males and 0.66-0.79 in females, a similar total body BMD of 0.981 g/cm^2 and total body BMD Z-scores ranging from -1.8 to 1.0 (52).

Another recent study of SRS, which included 29 individuals with SRS (20 molecularly confirmed), showed that lean body mass: was lower in SRS than non-SRS SGA; stayed the same during treatment; and declined after GH treatment ended but then stabilised after 18 months. This study reported lean body mass and fat mass percentage in terms of SDS and absolute numbers not reported therefore could not directly be compared with this cohort (80). There are no studies known to this author reporting fat-free mass index or lean mass index in SRS.

7.5.1.2 Metabolism and cardiovascular risk factors

The median cholesterol in this study was 4.90 mmol/L with levels above the recommended 5 mmol/L in 52.0%. In individuals aged ≥18 years, high LDL cholesterol was found in 39.1% and high triglyceride levels in 16.1%. These are established risk factors for cardiovascular disease suggesting that adults with SRS are at an increased risk.

Elevated glucose levels were seen in 25.0% of individuals ≥18 years in this cohort and this included impaired fasting glycaemia as well as diabetes mellitus. The prevalence of diabetes mellitus alone was 12.5%. This is lower than the 2014 World Health Organization reported prevalence of 'hyperglycaemia' in the UK, which was 15.2% of the population aged ≥18 years (using the same criteria as used as in this study for diabetes: blood glucose ≥7.0 mmol/L or on medication for raised blood glucose or with a history of diagnosis of diabetes) (310).

The prevalence of metabolic syndrome was 15.4% in the overall cohort and 18.2% in those aged ≥18 years. This is lower than the International Diabetes Federation estimate of 25% worldwide prevalence in adults (307). However, there are few adults aged ≥50 years in this cohort and the difference might result from different age distributions. Prevalence of hypertension in the overall cohort was 27.6%; in individuals aged ≥18 years only 33.3%. The overall prevalence is slightly higher than the World Health Organization reported prevalence of hypertension in UK, which was

22.6% (using comparable criteria for diagnosis: raised systolic BP ≥140 mmHg or diastolic BP ≥90 mmHg).

The median insulin resistance measured by HOMA-IR was 1.05 in individuals aged ≥18 years and 0.958 in those aged <18 years. These values are both lower than the median insulin resistance of 1.21 in normal subjects (240) reflecting normal insulin resistance in this cohort. The median results for QUICKI were 0.383 in individuals aged ≥18 years and 0.386 in those aged <18 years. These are both greater than the mean value of 0.366 seen in healthy adults and the proposed limit value of 0.357 (311) reflecting normal insulin sensitivity in this cohort.

Several results observed in this study are supported by the findings of the case series of seven individuals with molecularly confirmed SRS (52): the median cholesterol level observed in this study was within the range reported, which was 4.13 to 5.92 mmol/L. Total cholesterol was high in 52.0% of those aged ≥18 years in this study compared to 2/7 (28.6%) cases of hypercholesterolaemia in the case series, although the limit used was 5.17 mmol/L compared to 5.0 mmol/L in this study. A limit of 5.0 mmol/L would have diagnosed 3/7 (42.9%) cases with hypercholesterolaemia which is closer to the prevalence seen in this study. The median LDL of 2.55 mmol/L in this study is within the range of 1.81 to 4.39 mmol/L reported. The median fasting glucose of 4.7 mmol/L seen in this study was similar to the levels of 4.4 to 5.4 mmol/L observed. However, there are some results which contrast with the findings of the Patti et al (2018) case series: high LDL cholesterol (4/7;57.1%) and low HDL cholesterol (4/7;57.1%) were observed in the case series but not in this study. Hypertriglyceridaemia was observed in this study but normal triglycerides were reported in all seven cases in the series. Fasting insulin levels in this study were lower at 3.60 mU/L than 4.3 to 20.5 mU/L. Of the six individuals with reported blood pressure measurements in the case series, none were diagnosed with hypertension (diagnostic criteria: systolic BP ≥135 mmHg and/or diastolic BP ≥85mmHg). These criteria were similar to the criteria in this study (systolic BP ≥130 mmHg and/or diastolic BP ≥85mmHg) and application of the criteria used here would not change the prevalence of hypertension in the case series. No cases of metabolic syndrome or diabetes mellitus were diagnosed in the series (52).

The larger study including 29 individuals with SRS (20 molecularly confirmed diagnoses) reported (80): similar LDL cholesterol levels with mean levels at baseline and two years following treatment of 2.3 mmol/L and 2.7 mmol/L respectively; similar HDL cholesterol levels, with mean levels at baseline and two years following treatment, of 1.3 mmol/L and 1.4 mmol/L respectively; and comparable fasting blood glucose concentrations of 4.0 mmol/L and 5.0 mmol/L. However, there were some contrasting results reported in that study: lower triglyceride levels of 1.1 mmol/L; lower mean cholesterol levels of 4.1 mmol/L at baseline and 4.4 mmol/L two years after GH treatment discontinuation; and higher mean insulin concentrations of 9.4 and 13.8 mU/L. No

cases of metabolic syndrome were diagnosed in that study and although fasting blood glucose concentration increased on GH treatment and remained high after two years, type 2 diabetes mellitus was not demonstrated (80).

Although there were similarities between this study and the two discussed above, there are also differences. This study included a greater number of individuals, exclusively included molecularly confirmed SRS cases and included older individuals. These factors might account for some observed differences and strengthen the results presented.

7.5.2 Differences in body composition, metabolic problems and cardiovascular risk factors between adults and adolescents with SRS

There was a higher prevalence of underweight and no obesity in the younger age group. Waist-to-hip ratios and body fat were lower in adolescents. These results suggest that body composition changes over time in SRS. This pattern is similar to that seen in the general population with abdominal adiposity increasing with age. This finding in SRS is compatible with the increased cardiovascular disorders seen in children born SGA.

Grip strength was lower in individuals aged ≥18 years than those aged <18 years, suggesting reduced muscle strength in older individuals with SRS. The difference in muscle strength may have resulted from differences in muscle such as mass, cross-sectional area or the balance of muscle fibre types (type I slow twitch muscle fibres are weaker; type II fast-twitch fibres are stronger).

FFMI (BIA) was higher in those aged ≥18 years than those aged <18 years, which results from increased lean muscle mass and/or increased BMD. The finding of reduced grip strength in the older age group might suggest the higher FFMI did not result from increased muscle mass.

The higher FFMI may have resulted from increased BMD which is supported by the finding that total BMD and spine BMAD were both significantly greater in individuals ≥18 years. However, grip strength has been to shown to increase with age to a point and then decrease. The group of individuals ≥18 years included a wide age range therefore this may have influenced the results obtained.

GGT levels were higher in individuals ≥18 years than those aged <18 years in the study. These were all found in females, and with raised BMI SDS in two out of three cases. Elevated ALT was found in only one case who was known to have non-alcoholic steatohepatitis. Two of these individuals also reported hypothyroidism and the third reported a history of anorexia nervosa and bulimia. The effects of hypothyroidism on liver function are unclear. There are reports of

abnormal liver function tests, however those reported were in association with concomitant abnormal thyroid function (312, 313)). To the author's knowledge, there are no studies on hypothyroidism and liver function in SRS. In the cases seen in this study, thyroid function tests were normal therefore this aetiology is unlikely. Abnormal GGT and ALT can be observed in anorexia nervosa however have been shown to negatively correlate with adiposity (314) whereas the individual with this history had a BMI SDS of 2.68. Alcohol intake was not reviewed during the study appointment and is a probable confounding factor.

IGF1 levels were higher in adolescents than adults however IGF1 SDS were similar. This suggests that the difference in absolute IGF1 levels reflected an age effect. The higher levels in adolescents would also have been affected by the four individuals aged <18 years who were receiving GH treatment at the time of the blood sampling.

7.5.3 The effects of GH on body composition, metabolic problems and cardiovascular risk factors in SRS

7.5.3.1 Body composition

In the overall group, GH treatment was associated with improved weight status; increased prevalence of ideal weight, reduced obesity but also a reduced number of people being in the underweight category. SFT body fat percentage and BIA FMI were higher in GH-untreated than GH-treated individuals. Triglyceride levels were higher in GH-untreated individuals which supports the increased fat storage demonstrated in the results presented above. Between the GH treatment groups in individuals aged ≥18 years, there were no differences in BMI SDS, body fat percentage, FMI, FFMI, LMI or grip strength SDS.

Spine BMAD was greater in GH-untreated individuals although neither the difference in Z-score in the cohort overall nor the difference when only those aged ≥18 were included, reached statistical significance. This result overlaps with the finding that older individuals had a greater total BMD and spine BMAD. The increased BMAD might result from increased weight and therefore increased load bearing in the GH-untreated group. However, total body BMD but not spine BMAD positively correlated with BMI SDS which might reflect differential effects of weight as a load bearing force. Weight and BMI both positively correlate with bone mass (315-318) although osteopenia and osteoporosis are reported despite obesity in some cohorts (319, 320). BMD correlated with body fat percentage but not lean mass and did not correlate with time since GH discontinuation.

In the overall STAARS UK cohort (n=33), there was a suggestion that BMI was higher in GH-untreated individuals than in GH-treated individuals (median BMI SDS 0.57 and -1.09

respectively, p=0.155). However, the results of the STAARS collaboration cohort (n=71) showed that BMI was higher in GH-untreated individuals compared to GH-treated (see Chapter 6). Whilst it is possible that the heterogeneity of the European cohort affected the results obtained, causing a type I error, the results from the STAARS UK subgroup support the results from the STAARS collaboration cohort. It is possible that the lower number (n=22) in the group aged ≥18 years for whom body composition data was available, would result in type II errors because this aspect of the study is likely to be underpowered.

7.5.3.2 Metabolism and cardiovascular risk factors

In individuals aged ≥18 years, triglyceride levels were significantly higher in GH-untreated individuals than in GH-treated and the prevalence of metabolic syndrome was greater in GH-untreated individuals than GH-treated individuals although this did not reach statistical significance. Elevated triglyceride levels are associated with increased cardiovascular risk (321, 322) and metabolic syndrome is associated with a two-fold increased risk of cardiovascular disease (323). These findings therefore suggest that individuals with SRS aged ≥18 years have an increased risk of cardiovascular problems.

7.5.4 Limitations

DXA is widely acknowledged as the gold standard for assessment of body composition, however, in this study a greater number of differences were demonstrated using SFTs and BIA: the strongest correlation between BMI SDS and body fat percentage was seen with SFT measurements; the strongest correlation of FMI with BMI SDS was seen with DXA in the cohort overall and with BIA in individuals aged ≥18 years old only. DXA results are known to be affected in short stature therefore adjustments can be made to convert areal BMD to BMAD. It is possible that adjustments could be made for short stature in the assessment of body composition. It is outside the scope of this study to assess the optimal method for evaluation of body composition in SRS as a greater number of individuals would be required in the cohort.

The possibility of observer bias – whereby the researcher has a tendency to observe what he/she expects or wishes to see – is present with the results obtained, particularly in the case of SFT measurements. However, observer bias would not have affected BIA results which supported those obtained by SFT measurement.

During puberty, peak bone accretion occurs in both sexes, females gain fat mass, and males gain skeletal mass and fat free mass (324). The individuals aged <18 years may not all have completed

puberty, which would affect their body composition and is a limitation to the interpretation of the results in the overall cohort and <18 years subgroup.

Despite statistically significant results, the possibility remains that these could have been obtained by chance. Type I errors or false positives would result in declaring a significant difference even when the null hypothesis is true. When a significance level of 0.05 is set (i.e. $p \leq 0.05$ significant; $p > 0.05$ not significant), the type I error rate is fixed at 0.05 (i.e. 5%). Thus, even when the null hypothesis is true, extreme values will be obtained at the tails of distributions. However, when a value in the outer 5% is seen, a significant difference is declared. By definition, this will happen 5% of the time (i.e. one in 20 times) when the null hypothesis is true.

The Bonferroni correction/Bonferroni adjustments can be applied when several dependent or independent statistical tests are being performed simultaneously on a single data set and deflates the significance level applied to each test such that the overall error rate remains at 0.05. This means that for 20 tests the adjusted significance level would be 0.0025 for each test.

Type II errors or false negatives would result in accepting a null hypothesis as a result of a non-significant difference when groups are actually different (as a result of insufficient data). Type II error relates to power (=100 minus power); if a study has 80% power to detect a difference then the type II error rate is 20%. It is not possible to eliminate the two types of error and for a given sample size, they are balanced against one another.

Problems with Bonferroni adjustments have been highlighted (325). These included, firstly, rejection of the universal null hypothesis if one or more of the adjusted P values is significant (i.e. the result does not inform us as to which one is significant). Secondly, Bonferroni adjustments suggest that the interpretation of a given comparison will be different depending on how many tests are performed (e.g. treatment success rates would be interpreted differently if survival rates, complication rates and quality of life scores were also assessed). Thirdly, an increase in type II errors will occur in order to reduce type I errors. As type II errors are no less false than type I errors, this does not guarantee a wise interpretation of results. Finally, the decision about when to apply Bonferroni adjustments presents difficulties and it has been suggested that Bonferroni adjustments are not useful when specific hypotheses are being tested (325). Bonferroni adjustments have not been applied to the statistical analysis in this thesis. However, the results could be viewed as exploratory and should be subjected to confirmatory research.

The metabolic results presented in this thesis appear to fit together in a coherent manner: GH treatment in SRS is associated with improved weight status, lower body fat and lower triglyceride levels.

7.5.5 Further work

Although 68% of this overall cohort had a low serum creatinine, it was neither a sensitive biochemical marker for low muscle mass nor in this study, able to discern differences between GH treatment groups. A recent study demonstrated creatinine levels were significantly lower in SRS than non-SRS SGA but within the normal range (80). Further work could be done to examine creatinine levels in a greater number of GH-untreated and GH-treated individuals or alternative biochemical markers of muscle mass.

7.5.6 Conclusions

The results presented in this chapter show that weight status in SRS reflects the general population in terms of overweight and obesity but that a high proportion of individuals are underweight. This study has demonstrated that weight status and body composition change with age in SRS with higher body fat in adulthood but not adolescence, and that GH treatment was associated with improved weight status.

Chapter 8 Wellbeing, disability and quality of life in SRS

8.1 Evaluation of wellbeing in SRS

The results presented in this chapter relate to the molecularly confirmed SRS cases of the UK STAARS cohort. Results are reported for this cohort overall (n=33) and separately for individuals aged ≥18 years (n=25).

8.1.1 Wellbeing in overall cohort

The methodology used to assess wellbeing is described in section 3.8.1. Participants were asked to describe their health, current feelings about their school/job, and position on a life satisfaction ladder. Participants were also asked the extent to which they agreed or disagreed with three statements (feeling like an outsider or left out, feeling awkward and out of place, and feeling lonely).

In the cohort overall, the median score of the life satisfaction ladder was 8.0 out of 10.0 (IQR 7.0-8.0). Table 8.1 to

Table 8.5 show the results of the descriptions of health, current 'feeling about school/job', 'feeling like an outsider or left out', 'feeling awkward and out of place', and 'feeling lonely'.

Table 8.1 Descriptions of health. Data presented as percentage with numbers in parentheses.

Description of health	UK STAARS cohort	UK STAARS cohort aged ≥18 years
Excellent	21.2% (7/33)	16.0% (4/25)
Good	45.5% (15/33)	48.0% (12/25)
Fair	21.2% (7/33)	24.0% (6/25)
Poor	12.1% (4/33)	12.0% (3/25)

Table 8.2 Descriptions of feelings regarding place of education or employment. Data presented as percentage with numbers in parentheses.

Current feeling about school/job	UK STAARS cohort	UK STAARS cohort aged ≥18 years
I like it a lot	55.2% (16/29)	66.7% (14/21)
I like it a bit	34.5% (10/29)	28.6% (6/21)
I do not like it very much	10.3% (3/29)	4.8% (1/21)
I do not like it all	0%	0%

Table 8.3 Descriptions of feelings of isolation. Data presented as percentage with numbers in parentheses.

'I feel like an outsider or left out'	UK STAARS cohort	UK STAARS cohort aged ≥18 years
Strongly agree	6.1% (2/33)	8.0% (2/25)
Agree	27.3% (9/33)	24.0% (6/25)
Disagree	30.3% (10/33)	28.0% (7/25)
Strongly disagree	36.4% (12/33)	40.0% (10/25)

Table 8.4 Descriptions of feelings of being discomfited. Data presented as percentage with numbers in parentheses.

'I feel awkward and out of place'	UK STAARS cohort	UK STAARS cohort aged ≥18 years
Strongly agree	6.1% (2/33)	8.0% (2/25)
Agree	27.3% (9/33)	24.0% (6/25)
Disagree	42.4% (14/33)	40.0% (10/25)
Strongly disagree	24.2% (8/33)	28.0% (7/25)

Table 8.5 Descriptions of loneliness. Data presented as percentage with numbers in parentheses.

'I feel lonely'	UK STAARS cohort	UK STAARS cohort aged ≥18 years
Strongly agree	3.0% (1/33)	0%
Agree	15.2% (5/33)	20.0% (5/25)
Disagree	42.4% (14/33)	40.0% (10/25)
Strongly disagree	39.4% (13/33)	40.0% (10/25)

8.1.2 Wellbeing in individuals aged ≥18 years

In those aged ≥18 years, the median score of the life satisfaction ladder was also 8.0 (IQR 7.0-8.0).

Table 8.1 to

Table 8.5 show the results of the descriptions of health, current 'feeling about school/job', 'feeling like an outsider or left out', 'feeling awkward and out of place', and 'feeling lonely'.

8.1.3 Wellbeing in SRS and the effects of growth hormone treatment

8.1.3.1 Overall cohort

There was no difference between GH-untreated (n=10) and GH-treated (n=23) individuals in their life satisfaction ladder scores; 7.5 (IQR 4.0-8.4) and 8.0 (IQR 7.0-8.0) respectively (p=0.340) (Figure 8.1), although the range of answers was greater in the untreated group. Neither were there differences in: their descriptions of health (p=0.655) (Figure 8.2), feelings about school/job (p=0.573) (Figure 8.3), feelings of being an outsider or left out (p=0.899) (Figure 8.4), feeling awkward and out of place (p=0.488) (Figure 8.5) or feeling lonely (p=0.771) (Figure 8.6).

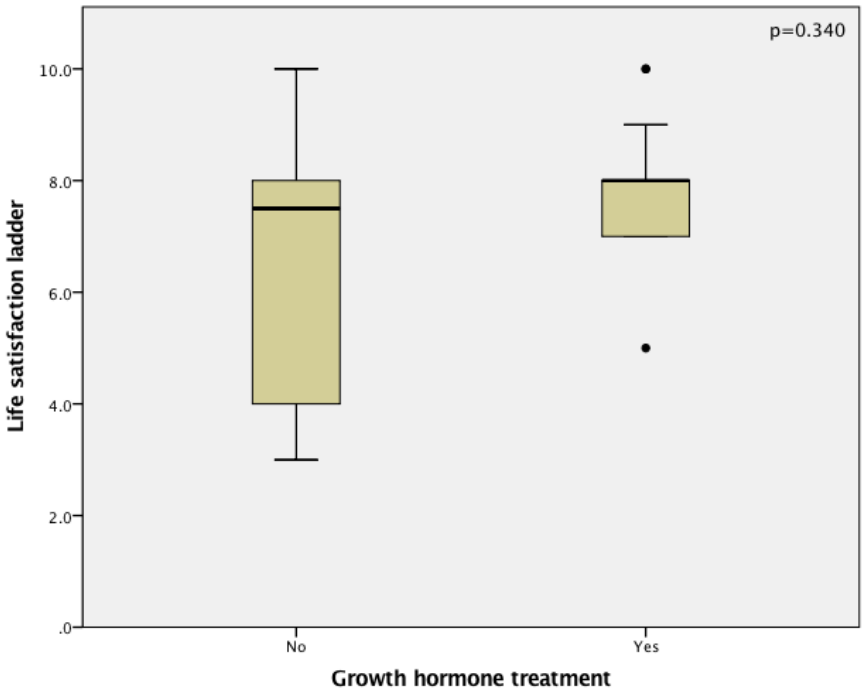


Figure 8.1 Results of life satisfaction ladder scores in the overall cohort (aged 13.36-69.71 years, median 29.58) separated by growth hormone treatment group. P value shown for comparison of life satisfaction scores between GH treatment group.

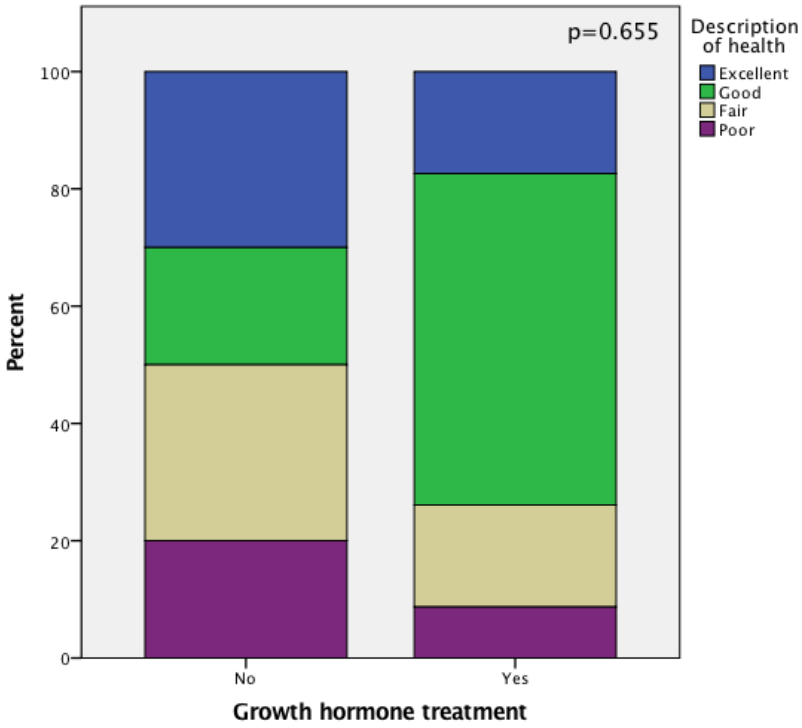


Figure 8.2 Descriptions of perceived health in the overall cohort separated by growth hormone treatment group. P value shown for comparison of distribution of responses between GH treatment groups.

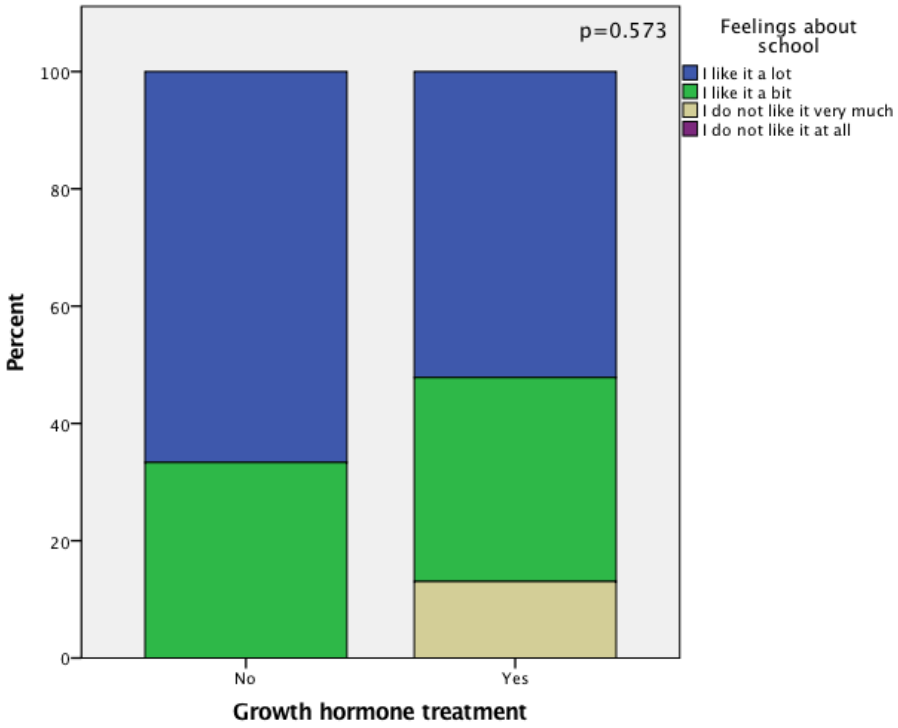


Figure 8.3 Feelings regarding school/job in the overall cohort separated by growth hormone treatment group. P value shown for comparison of distribution of responses between GH treatment groups.

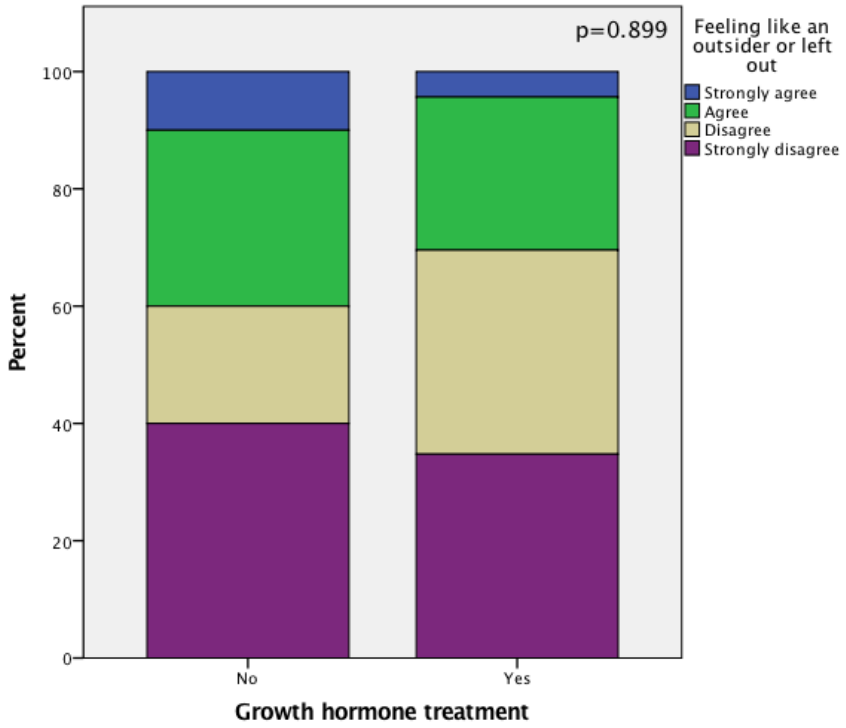


Figure 8.4 Feelings of exclusion in the overall cohort separated by growth hormone treatment group. P value shown for comparison of distribution of responses between GH treatment groups.

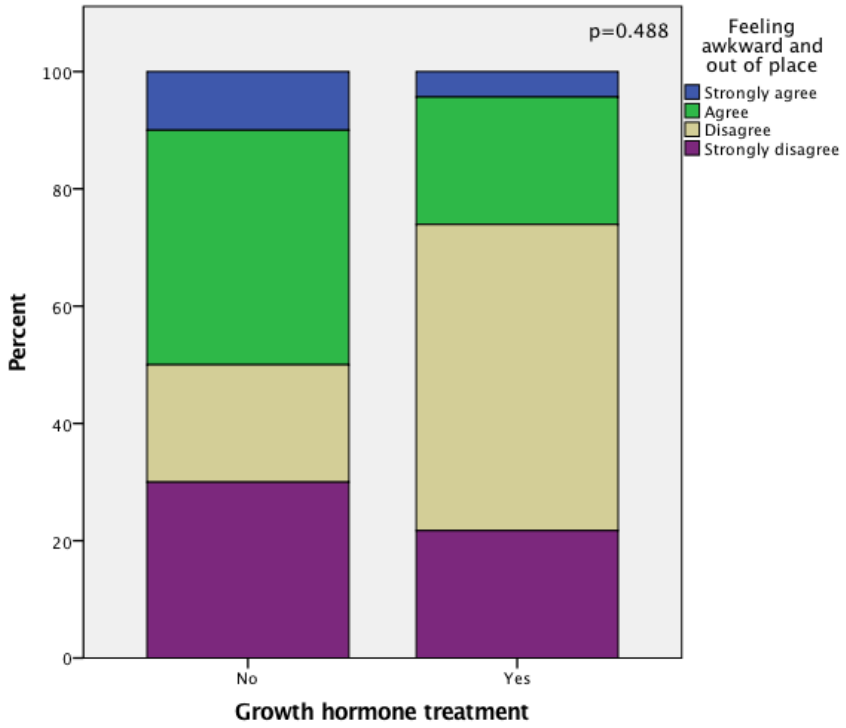


Figure 8.5 Feelings of being discomfited in the overall cohort separated by growth hormone treatment group. P value shown for comparison of distribution of responses between GH treatment groups.

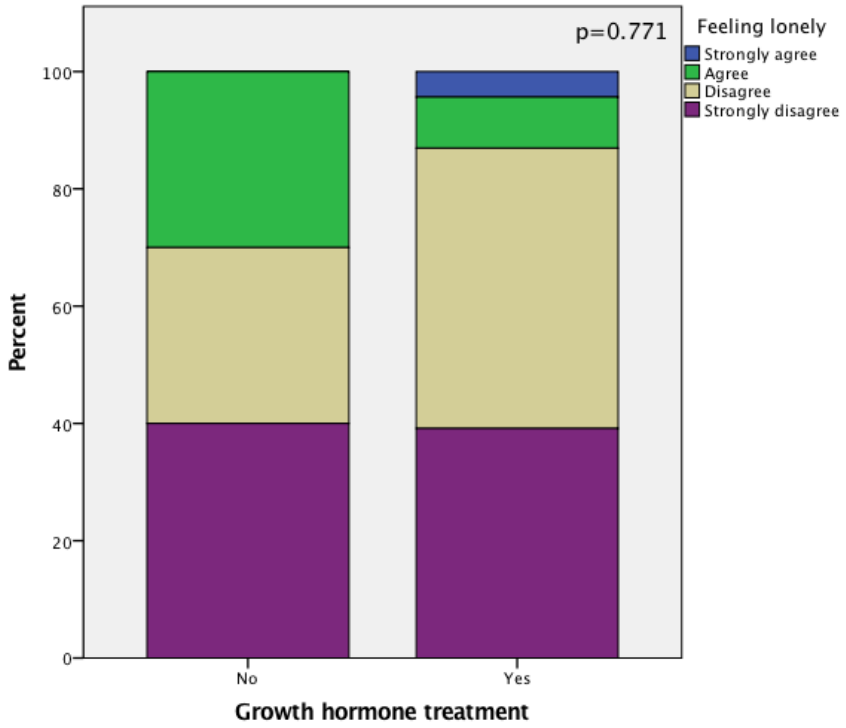


Figure 8.6 Feelings of loneliness in the overall cohort separated by growth hormone treatment group. P value shown for comparison of distribution of responses between GH treatment groups.

8.1.3.2 Individuals aged ≥18 years

In those aged ≥18 years, there was no difference between GH-untreated (n=10) and GH-treated (n=15) individuals in their life satisfaction ladder scores;7.5 (IQR 4.0-8.4) and 8.0 (IQR 7.0-8.0) respectively (p=0.418) (Figure 8.7). There were also no differences in: their descriptions of health (p=0.789) (Figure 8.8), feelings about school/job (p=0.970) (Figure 8.9), feelings of being an outsider or left out (p=0.762) (Figure 8.10), feeling awkward and out of place (p=0.489) (Figure 8.11) or feeling lonely (p=0.783) (Figure 8.12).

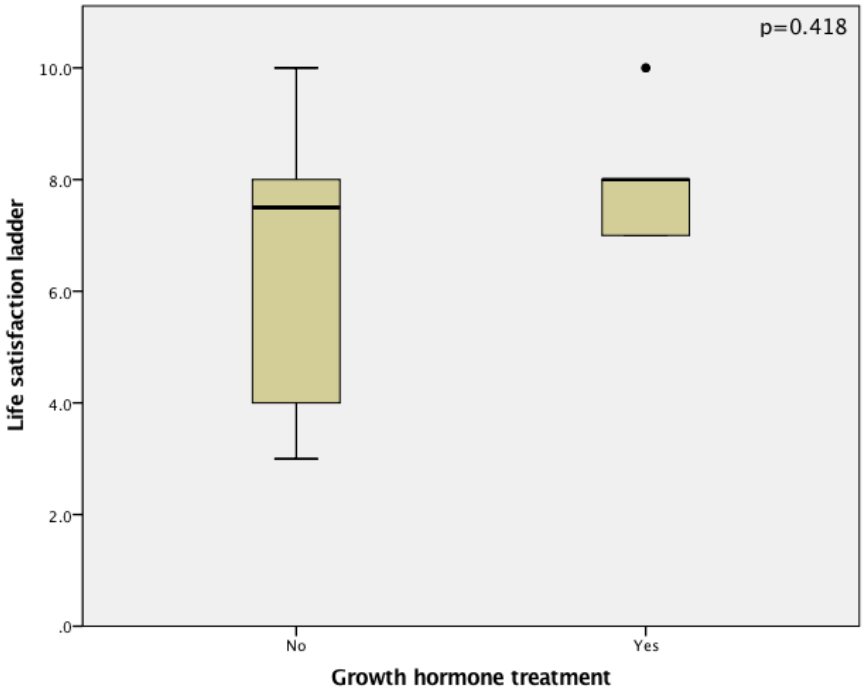


Figure 8.7 Results of life satisfaction ladder scores in individuals aged ≥ 18 years separated by growth hormone treatment group. P value shown for comparison of distribution of life satisfaction scores between GH treatment groups.

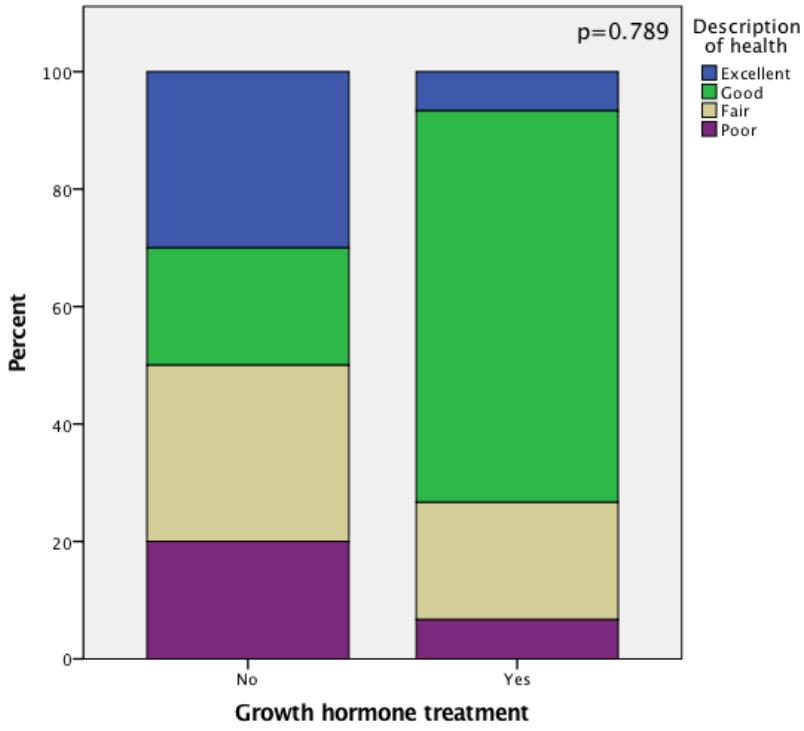


Figure 8.8 Descriptions of perceived health in individuals aged ≥ 18 years separated by growth hormone treatment group. P value shown for comparison of distribution of responses between GH treatment groups.

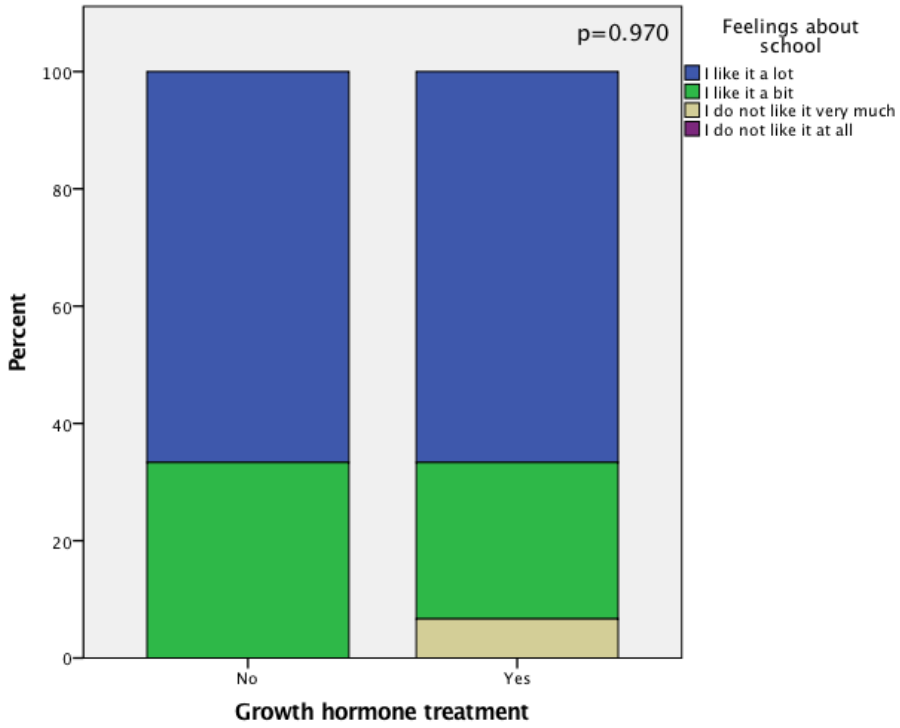


Figure 8.9 Feelings regarding school/job in individuals aged ≥ 18 years separated by growth hormone treatment group. P value shown for comparison of distribution of responses between GH treatment groups.

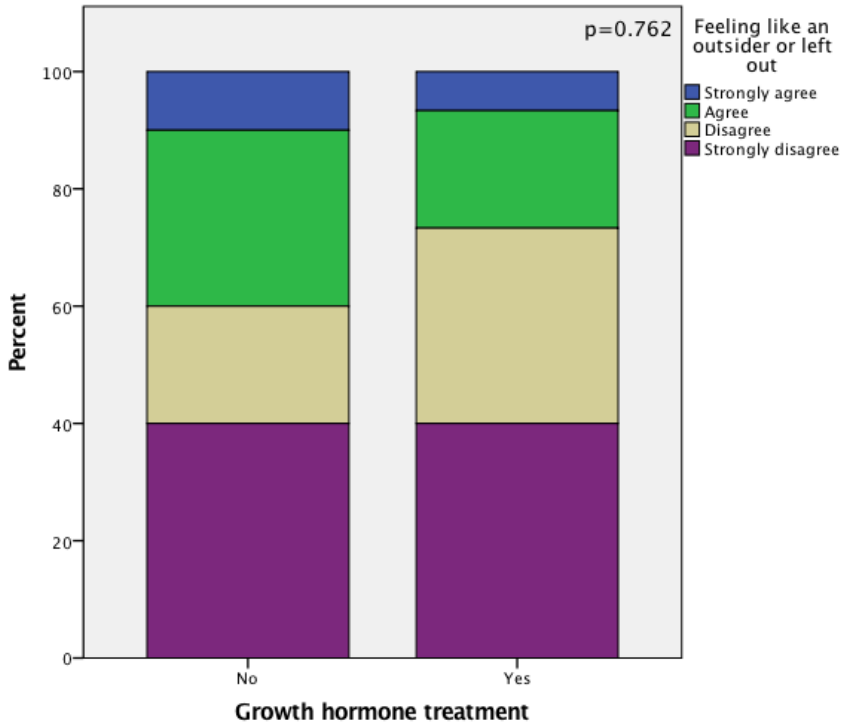


Figure 8.10 Feelings of exclusion in individuals aged ≥ 18 years separated by growth hormone treatment group. P value shown for comparison of distribution of responses between GH treatment groups.

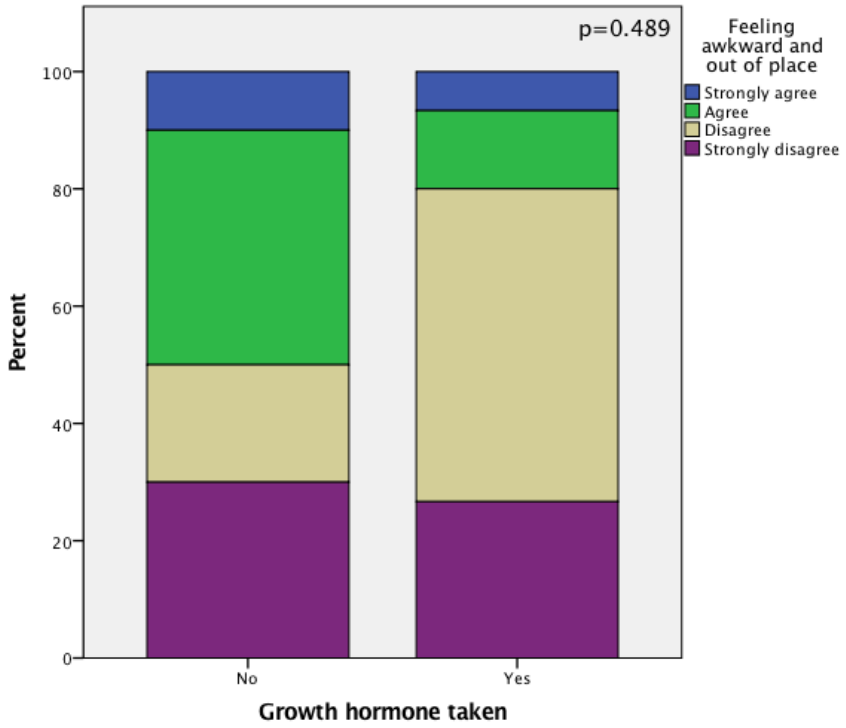


Figure 8.11 Feelings of being discomfited in individuals aged ≥ 18 years separated by growth hormone treatment group. P value shown for comparison of distribution of responses between GH treatment groups.

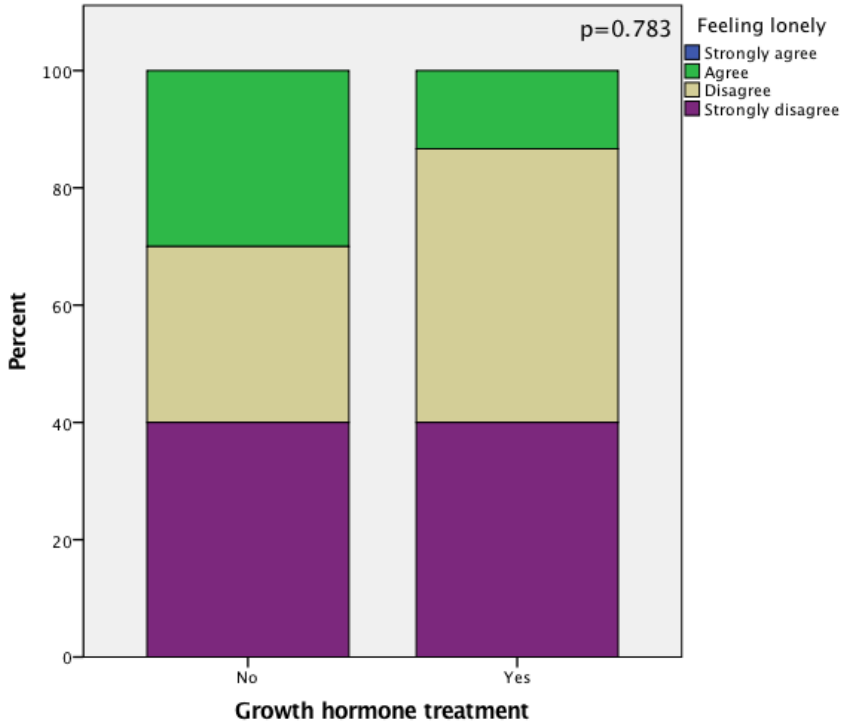


Figure 8.12 Feelings of loneliness in individuals aged ≥ 18 years separated by growth hormone treatment group. P value shown for comparison of distribution of responses between GH treatment groups.

8.2 Evaluation of disability in SRS

8.2.1 Disability in SRS

The Sheehan Disability Scale was administered as described in section 3.8.3. Each participant was asked about how living with SRS affected his/her life over the preceding week. Each subscale was graded from 0 to 10 with 0 reflecting not feeling disabled and 10 reflecting feeling extremely disabled. The total score therefore ranges from 0 to 30 with lower scores reflecting mild levels of disability; and higher scores severe levels of disability.

8.2.1.1 Overall cohort

The results from the Sheehan Disability Scale assessment are shown in Table 8.6. In the cohort overall, the median total Sheehan Disability Scale score was 3 out of 30 and 33.3% gave a score of 0. In relation to disruption of job/volunteering/school, the median score was 1 out of 10 and 40.7% (11/27) gave a score of 0. The median score for disruption to social life and leisure activities was 0 out of 10 and 51.5% (17/33) gave a score of 0. The median score for disruption to

family life and responsibilities at home was 0 out of 10 and 66.7% gave a score of 0. The median number of days lost was 0 with 84.8% reporting this number. The median number of unproductive days was 0 with 75.8% reporting this number.

Table 8.6. Scores of Sheehan Disability Scale. Data presented as median (IQR) with number, n shown in parentheses.

	UK STAARS cohort	UK STAARS cohort aged ≥18 years
Total score of Sheehan Disability Scale	3 (0-14) (n=27)	1 (0-11.5) (n=21)
Score for disruption to job/volunteering/school	1 (0-4) (n=27)	1 (0-4.5) (n=21)
Score for disruption to social life and leisure activities	0 (0-3) (n=33)	0 (0-3.5) (n=25)
Score for disruption to family life and home responsibilities	0 (0-3.5) (n=33)	0 (0-1) (n=25)
Number of days lost	0 (0-0) (n=33)	0 (0-0) (n=25)
Number of days unproductive	0 (0-0.5) (n=33)	0 (0-0) (n=25)

In the cohort overall, there were no significant correlations between total Sheehan Disability Scale score and 1) height SDS (correlation coefficient -0.172, p=0.392); 2) weight SDS (correlation coefficient 0.261, p=0.188; 3) BMI SDS (correlation coefficient 0.307, p=0.119). There were no significant differences in Sheehan Disability Scale score between individuals with asymmetry and those without in overall score (p=0.449) or scores for disruption to job/volunteering/school (p=0.132), disruption to social life and leisure activities (p=0.836), disruption to family life and home responsibilities (p=0.721), days lost (p=0.721), and unproductive days (p=0.807).

8.2.1.2 Individuals aged ≥18 years

The results from the Sheehan Disability Scale assessment in individuals aged ≥18 years are shown in Table 8.6. The median total Sheehan Disability Scale score was 1 out of 30 with 14.3% reporting this number and 38.1% reporting a score of 0. In relation to disruption of job/volunteering/school, the median score was 1 out of 10 with 14.3% reporting this number and 47.6% reporting a score of 0. The median score for disruption to social life and leisure activities was 0 out of 10 with 60.0% reporting this number. The median score for disruption to family life and responsibilities at home was 0 out of 10 with 72.0% reporting this number. The median number of days lost was 0 with 88.0% reporting this number. The median number of unproductive days was 0 with 80% reporting this number.

In individuals aged ≥18 years, there were no significant correlations between total Sheehan Disability Scale score and 1) height SDS (correlation coefficient -0.201, p=0.381); 2) weight SDS (correlation coefficient 0.317, p=0.162). There was a positive correlation between BMI SDS and Sheehan Disability Scale score (correlation coefficient 0.431, p=0.051). There were no significant differences in Sheehan Disability Scale score between individuals with asymmetry and those without in overall score (p=0.535) or scores for disruption to job/volunteering/school (p=0.224), disruption to social life and leisure activities (p=0.803), disruption to family life and home responsibilities (p=0.718), days lost (p=0.978), and unproductive days (p=0.846).

8.2.2 Disability in SRS and the effects of growth hormone treatment

8.2.2.1 Overall cohort

In the overall cohort, there were no significant differences between GH-untreated and GH-treated for the following parameters: total Sheehan Disability Scale score (median scores 5.5 and 3.0 respectively, p=0.341); disruption of job/volunteering/school (median scores 1.0 and 1.5, p=0.685); disruption to social life and leisure activities (median scores 0.5 and 0.0, p=0.576); disruption to family life and responsibilities at home (median scores 0 and 0.0, p=0.630); number of days lost (median number of days 0 and 0, p=0.773); number of unproductive days (median number of days 0 and 0, p=0.603).

8.2.2.2 Individuals aged ≥18 years

In individuals aged ≥18 years, there were no significant differences between GH-untreated and GH-treated for the following parameters: total Sheehan Disability Scale scores (median scores 9 and 1 respectively, p=0.287); disruption of job/volunteering/school (median scores 1 and 0.5, p=0.535); disruption to social life and leisure activities (median scores 0.5 and 0, p=0.397);

disruption to family life and responsibilities at home (median scores 0 and 0, p=0.397); number of days lost (median number of days 0 and 0, p=0.605); number of unproductive days (median number of days 0 and 0, p=0.461).

8.3 Quality of life in SRS

8.3.1 Evaluation of quality of life in SRS

8.3.1.1 Overall cohort

Schedule for the evaluation of individual quality of life – direct weighting (SEIQoL-DW) was administered as described in section 3.8.2. In the overall cohort, the mean SEIQoL-DW index score was 74.9 out of maximum of 100 (SD 13.0, n=31). There was no significant correlation between SEIQoL-DW index score and height SDS (Pearson correlation coefficient 0.117, p=0.529). There was a negative correlation between SEIQoL-DW index score and BMI SDS (Pearson correlation coefficient -0.388, p=0.031) (Figure 8.13). There was a suggestion of a negative correlation between SEIQoL-DW index score and weight SDS (Pearson correlation coefficient -0.329 (p=0.071).

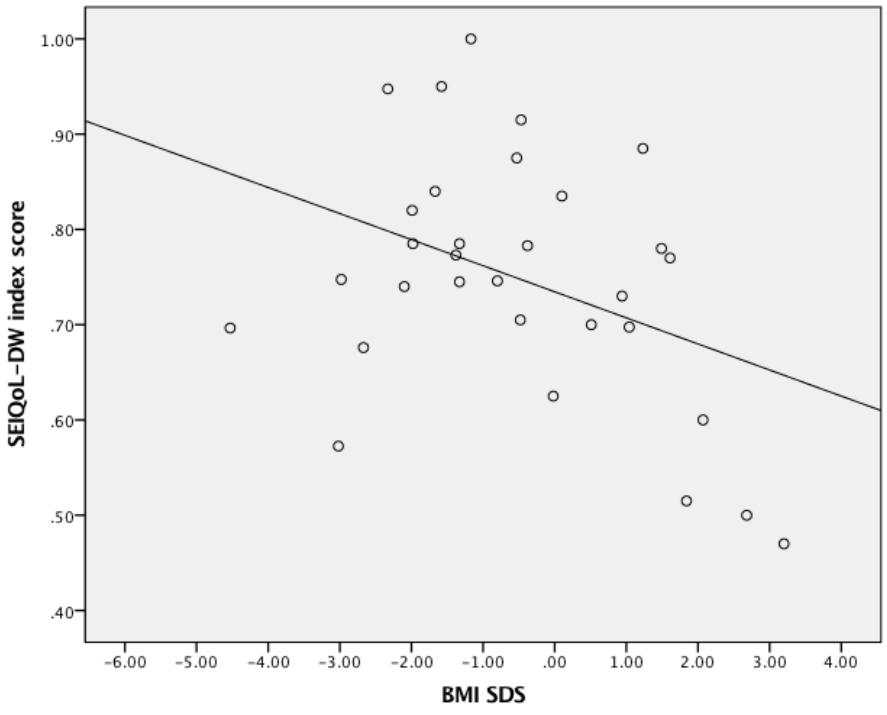


Figure 8.13 Scatter plot of SEIQoL-DW index score against BMI SDS. Line of best indicated.

8.3.1.2 Individuals aged ≥18 years

In individuals aged ≥18 years, the mean SEIQoL-DW index score was 72.8 (SD 14.3). There was no significant correlation between SEIQoL-DW index score and height SDS (Pearson correlation coefficient -0.004, p=0.984). There was a negative correlation between SEIQoL-DW index score and BMI SDS (Pearson correlation coefficient -0.454, p=0.03). There was a negative correlation between SEIQoL-DW index score and weight SDS (Pearson correlation coefficient -0.453, p=0.03).

8.3.2 Quality of life in SRS and the effects of growth hormone treatment

In the cohort overall, the mean SEIQoL-DW index scores in GH-untreated and GH-treated groups were 69.3 (SD 17.0) and 77.6 (SD 10.1) respectively (p=0.177). In individuals aged ≥18 years, the mean SEIQoL-DW index scores in GH-untreated and GH-treated groups were 75.5 (SD 11.9) and 69.3 (SD 17.0) respectively (p=0.307).

8.4 Discussion

8.4.1 Life satisfaction in SRS

A Pubmed search for life satisfaction in SRS produced no published work. Achondroplasia is a condition which causes short stature – although with skeletal disproportion and with a lower mean adult height than seen in SRS. A Pubmed search for life satisfaction in achondroplasia yielded no results. The lack of published work in this aspect of SRS supports the need for the research presented here.

Overall life satisfaction was good in the cohort with a median self-reported score of 8.0 out of 10.0. The Cantril ladder of life measure has been used in international surveys and results summarised in the World Happiness report (326). The average UK score of 6.714 for the UK is below that seen in the UK STAARS cohort. Moreover, the average score of the highest ranked country was 7.537. In the context of these findings, the results in the UK STAARS cohort suggest that life satisfaction was above average. There is no immediate explanation of this finding. It may represent actual outcome compared to expectation in childhood. The findings may be related to the cohort of people engaged in the study who have chosen to take part and have a positive attitude to life or responded to please the interviewer. While this finding should be repeated in a bigger cohort, to the author's knowledge, life satisfaction has not previously been reported in SRS.

8.4.2 Wellbeing in SRS

Similarly to life satisfaction, the literature search did not identify any studies specifically on wellbeing in SRS. Neither were there studies on wellbeing in achondroplasia for comparison. The lack of published research in wellbeing in SRS substantiates the need for the work done in this study.

The results of the wellbeing questions showed that in the UK STAARS cohort, the majority of individuals described their health as 'good' and results were similar in the cohort overall and individuals aged ≥18 years only. The majority liked their place of education or employment 'a lot'. The majority strongly disagreed or disagreed that they: felt like an outsider or left out; felt awkward and out of place; and that they felt lonely. GH treatment was not associated with any differences in the above reports. These perceptions in SRS have not previously been reported to the author's knowledge and are different to some reports of reduced quality of life in individuals with short stature (209, 214, 327). The data presented here provide evidence that height and wellbeing are not necessarily associated in adult life. Perceived wellbeing may be more dependent on factors other than height – for example the presence or absence of health complications, support from friends and/or family, secure employment and financial stability could influence an individual's perception of wellbeing. The qualitative arm of the STAARS study explored the lived experience of people with SRS. Four themes were identified from participant interviews: 1) concerns relating to appearance rather than height alone; 2) resilience: coping strategies; 3) women's experience of disability and pain; 4) feeling disregarded in romantic relationships (221). Participants were reported feeling 'unwell' and tired. These themes show that psychological wellbeing is affected by factors other than height.

8.4.3 Disability in SRS

One of the themes identified in the qualitative arm of the STAARS study was disability, particularly in women with SRS. In addition, women reported pain with arthralgia and fatigue (221). No other studies on disability in SRS were identified during the literature search.

The median Sheehan Disability Scale scores were 3 (IQR 1 to 14) in the overall group and 1 (IQR 0 to 11.5) in individuals aged ≥18 years only. A score of 0 out of 30 would represent no disability whereas a score 30 out of 30 would represent high impairment. Intermediate cut-off values and definitions of severity have not been established, which makes it difficult to categorise the results in this study. However, these median values are at the very low end of the scale suggesting that this cohort did not report high levels of disability. The results showed that a median of 0 days were lost at work or school and 0 days were unproductive, which suggests that SRS did not cause

a significant burden in the STAARS UK cohort. Published studies using the Sheehan Disability Scale focus on psychiatric conditions and make comparisons between treatment groups or changes in scores over time.

Interestingly, there was no correlation between disability score and height which suggests that even the shortest heights in SRS did not confer a feeling of being disabled. This may have resulted from beneficial coping mechanisms or supportive factors. There was a positive correlation between BMI and disability in individuals aged ≥18 years. This increasing disability with increasing BMI may result from the physical effects of increased BMI for example difficulty in mobilising, arthralgia and myalgia. Increasing BMI and obesity specifically have been associated with declining physical function and increased disability (328-330) as well as increased bodily pain (328). Conversely, the presence of asymmetry was not associated with a difference in disability score, although increased pain could result from severe body asymmetry, particularly involving the spine as studies have shown that scoliosis is frequently accompanied by pain and may be associated with disability (331, 332). Previous GH treatment status was not associated with a difference in disability score. However, in some instances the number of individuals included in the UK STAARS cohort is insufficient to demonstrate differences which would be possible with a larger cohort (e.g. differences in BMI SDS were demonstrated in the larger collaborative STAARS cohort but not in the UK STAARS cohort alone).

Changes in the Sheehan Disability Scale score over time have been demonstrated to be useful and this could be an area for future work in larger SRS cohorts and as people with SRS are followed for longer.

8.4.4 Height and quality of life in SRS

The mean SEIQoL-DW index score was 74.9 which is comparable to that of 77.4 (SD 9.5) obtained in healthy adults (261), suggesting that in this cohort, quality of life was not impaired. This is in agreement with some studies which showed limited (333) or no support for the hypothesis that short stature socially or psychologically disadvantaged children (218, 334). The relationship between height and quality of life remains controversial with studies reporting lower quality of life in children with short stature (209, 327) and height as a significant predictor of health-related quality of life in a general population (214). However, other research has shown height is a weak predictor of health-related quality of life in a general population of adolescents (219). Adaptive processes are important in managing short stature (335). The qualitative arm of the STAARS study highlighted that factors other than height were of concern to individuals with SRS (221).

8.4.5 Body mass index and quality of life

To the author's knowledge, the negative correlation between BMI and quality of life (whereby quality of life reduces with increasing BMI) has not previously been reported in SRS.

In obesity, health-related quality of life is impaired (336) (337, 338). This particularly relates to the physical subdomain but emotional well-being is also affected (338). The increased pain and reduced mobility in obesity, discussed in section 8.4.3 in relation to disability, could also affect quality of life.

There may be similar underlying reasons in SRS, with increased physical problems associated with higher BMI affecting quality of life. BMI alone does not clarify the affected body compartment as increased muscle mass is less likely to cause reduced mobility; increased fat mass with reduced muscle mass is more likely to cause this. In older adults, both obesity and sarcopenic obesity are associated with lower health-related quality of life (339). Reduced muscle mass or muscle strength may be associated with impaired quality of life in SRS and this represents a potential area for further work.

8.4.6 Effects of GH treatment on quality of life in SRS

Interestingly, GH treatment status was not associated with a significant difference in quality of life. To the author's knowledge, there are no studies evaluating the effects of GH treatment on quality of life in SRS.

Prader-Willi syndrome (PWS) is another disorder of imprinting which is characterised by impaired growth, hypotonia with delayed motor development, and poor feeding in infancy. There is subsequently hyperphagia from approximately age 2 years. The typical behavioural profile includes resistance to change, stubbornness, controlling and compulsive behaviour (340). 8-38% have biochemical GH deficiency and treatment is positively associated with linear growth, body composition (improved lean body mass and body fat in both children and adults), metabolic homeostasis and cognitive function (341). GH treatment has been associated with increased health-related quality of life in PWS, in relation to the physical subdomain (342).

There are numerous reasons that GH treatment may not have been demonstrated to affect quality of life. Firstly, GH could truly not affect quality of life in SRS. There may be differing effects in SRS and PWS in the same way that fat mass has been shown to increase with GH in SRS (80) whereas in PWS fat mass reduces during GH treatment (341). Secondly, it is plausible that GH could affect quality of life but the numbers included here were insufficient to demonstrate this on significance testing. In the collaborative STAARS study, GH treatment was shown to affect

BMI (see section 6.2.7.2) and in this UK STAARS cohort, BMI was shown to affect quality of life. Therefore, it would follow that GH treatment could affect quality of life by affecting BMI. However, this finding is in agreement with another study of GH in short stature which did not demonstrate improved quality of life (343). Thirdly, GH treatment could transiently affect quality of life – during treatment or at a particular point in development – without long-term effects and therefore not be demonstrated in the retrospective study.

8.4.7 Limitations

There are a number of limitations in the in interpretation of this work. The responses were self-reported but via direct questioning so it is possible that responses could be different if given privately or anonymously. The individuals in the research study were self-selected, many of whom were members of a lay support group. This could reflect that they were more affected than others with SRS or have a more severe SRS phenotype and therefore engage with a supportive environment. Individuals presenting with SRS and those in contact with medical teams could have a higher prevalence or severity of medical problems or disabilities. These factors might affect the generalisability of research findings however this applies to much research in this field: a recent systematic review has highlighted the high risk of bias in existing research evaluating psychological outcomes in the treatment of short stature with GH (344). Finally, the number of individuals included in this analysis may not be sufficient to demonstrate significance in some areas.

8.4.8 Conclusions

These results represent an initial evaluation of wellbeing, disability and quality of life in SRS and suggest that most individuals are happy with their life and are not disabled by living with SRS. GH treatment did not affect life satisfaction or quality of life. The significant correlation of increasing BMI with reducing quality of life provides support for maintaining an ideal weight in SRS. This is potentially a modifiable factor and in addition to the well-recognised medical benefits, there could be a positive impact on quality of life that should be promoted to individuals with SRS.

Chapter 9 Conclusions

9.1 Conclusions

This thesis presented work on a research study evaluating long-term outcomes in SRS. Short stature and high body fat are important features of the adult phenotype of SRS. The adult phenotype differs from childhood descriptions and multiple medical problems appear in later life. The prevalence of respiratory disorders was marked. Arthralgia and/or myalgia affected females with SRS more than males. Despite early developmental delay, there is high educational attainment. The first hypothesis of the study was that the adult phenotype differs from the childhood phenotype, which has been demonstrated to be true.

GH-treated individuals with SRS were shorter in early life, gained more height and reached similar heights to GH-untreated individuals. A novel finding of lower BMI and lower BMI gain in association with GH treatment has been presented. This study has demonstrated that weight status and body composition change over time in SRS. Furthermore, this study has shown that GH treatment is associated with improved weight status, body fat and triglyceride levels. The second hypothesis of the study was that GH treatment increases final height and improves body composition in SRS. Total height gain was greater with GH treatment and it is likely that the without treatment the GH-treated group would have remained shorter that those untreated. Therefore, it is possible that GH increased the final height of those treated from what it otherwise would have been. GH treatment has not been shown to increase final height beyond those taller individuals who were untreated. Improved BMI, body weight status, body fat and triglycerides have been shown with GH treatment. These findings support the use of GH in SRS for linear growth and suggest there may be additional benefit to improve body composition and cardio-metabolic health.

Reducing quality of life with increasing BMI lends further support to the importance of maintaining an ideal weight in SRS. The final hypothesis of this study was that previous GH treatment is associated with improved quality of life. BMI has been shown to affect quality of life and disability, and GH treatment has been shown to affect BMI. However, a direct association between GH treatment and quality of life was not demonstrated.

9.2 Future work

The novel findings discussed in section 9.1 should be evaluated in other SRS cohorts. If these were replicated, further research would be warranted to understand the underlying aetiology.

Research collaboration would be a valuable method to establish larger cohorts of individuals with SRS with sufficient numbers to evaluate epigenotype-phenotype correlations in adults.

The changing eating patterns in individuals with SRS are of interest and an area of future work would be to document these. Subsequently, research into interventions to eating behaviours could be considered.

This study has shown the early and late BMI in SRS and the effects of GH treatment. Prospective evaluation of BMI and body composition over time and during GH treatment would delineate the growth pattern more clearly and might indicate a potential mechanism.

Appendix A List of publications from this research

I. Wakeling EL, Brioude F, Lokulo-Sodipe O, O'Connell SM, Salem J, Bliek J, Canton AP, Chrzanowska KH, Davies JH, Dias RP, Dubern B, Elbracht M, Giabicani E, Grimberg A, Grønskov K, Hokken-Koelega AC, Jorge AA, Kagami M, Linglart A, Maghnie M, Mohnike K, Monk D, Moore GE, Murray PG, Ogata T, Petit IO, Russo S, Said E, Toumba M, Tümer Z, Binder G, Eggermann T, Harbison MD, Temple IK, Mackay DJ, Netchine I. Diagnosis and management of Silver-Russell syndrome: first international consensus statement. Nat Rev Endocrinology. 2017;13(2):105 124. doi: 10.1038/nrendo.2016.138. Epub 2016 Sep 2.

The literature search conducted for this thesis and background on SRS contributed to the literature search for the above publication. Relevant articles were compiled and reviewed in order to refine the clinical diagnostic criteria for SRS and agree a consensus on the clinical diagnosis, molecular testing and management of SRS.

II. Ballard LM, Jenkinson E, Byrne CD, Child JC, Davies JH, Inskip H, Lokulo-Sodipe O, Mackay DJG, Wakeling EL, Temple IK, Fenwick A. Lived experience of Silver-Russell syndrome: implications for management during childhood and into adulthood. Arch Dis Child. 2018 Jun 28. doi: 10.1136/archdischild-2018-314952. [Epub ahead of print]

Of the participants recruited to the UK STAARS study and described in this thesis, a subgroup was subsequently involved in the qualitative research on the lived experience of SRS published in the study above. Data on molecular genetic diagnosis, height SDS and GH treatment from this thesis was included in the publication.

III. Begemann M, Rezwan FI, Beygo J, Docherty LE, Kolarova J, Schroeder C, Buiting K, Chokkalingam K, Degenhardt F, Wakeling EL, Kleinle S, González Fassrainer D, Oehl-Jaschkowitz B, Turner CLS, Patalan M, Gizewska M, Binder G, Bich Ngoc CT, Chi Dung V, Mehta SG, Baynam G, Hamilton-Shield JP, Aljareh S, Lokulo-Sodipe O, Horton R, Siebert R, Elbracht M, Temple IK, Eggermann T, Mackay DJG. Maternal variants in NLRP and other maternal effect proteins are associated with multilocus imprinting disturbance in offspring. J Med Genet. 2018 Jul;55(7):497-504. doi: 10.1136/jmedgenet-2017-105190. Epub 2018 Mar 24.

Clinical data was provided on two individuals from the UK STAARS study who are presented in this thesis for the above publication.

IV. Lokulo-Sodipe O, Canton APM, Giabicani E, Ferrand N, Child J, Wakeling EL, Binder G, Netchine I, Mackay DJG, Inskip HM, Byrne CD, Davies JH, Temple IK. Long term effects of childhood growth hormone treatment on height and body mass index in adolescents and adults with Silver-Russell syndrome. ESPE Abstracts (2018) 89 P-P1-181

Data on the European STAARS cohort, as included in this thesis, was prepared as an abstract for the European Society for Paediatric Endocrinology and subsequently delivered as a poster presentation.

Appendix B Publication I

Wakeling EL, Brioude F, Lokulo-Sodipe O, O'Connell SM, Salem J, Bliek J, Canton AP, Chrzanowska KH, Davies JH, Dias RP, Dubern B, Elbracht M, Giabicani E, Grimberg A, Grønskov K, Hokken-Koelega AC, Jorge AA, Kagami M, Linglart A, Maghnie M, Mohnike K, Monk D, Moore GE, Murray PG, Ogata T, Petit IO, Russo S, Said E, Toumba M, Tümer Z, Binder G, Eggermann T, Harbison MD, Temple IK, Mackay DJ, Netchine I. Diagnosis and management of Silver-Russell syndrome: first international consensus statement. Nat Rev Endocrinology. 2017;13(2):105 124. doi: 10.1038/nrendo.2016.138. Epub 2016 Sep

Appendix C Publication II

Ballard LM, Jenkinson E, Byrne CD, Child JC, Davies JH, Inskip H, Lokulo-Sodipe O, Mackay DJG, Wakeling EL, Temple IK, Fenwick A. Lived experience of Silver-Russell syndrome: implications for management during childhood and into adulthood. Arch Dis Child. 2018 Jun 28. doi: 10.1136/archdischild-2018-314952. [Epub ahead of print]

Appendix D Publication III

Begemann M, Rezwan FI, Beygo J, Docherty LE, Kolarova J, Schroeder C, Buiting K, Chokkalingam K, Degenhardt F, Wakeling EL, Kleinle S, González Fassrainer D, Oehl-Jaschkowitz B, Turner CLS, Patalan M, Gizewska M, Binder G, Bich Ngoc CT, Chi Dung V, Mehta SG, Baynam G, Hamilton-Shield JP, Aljareh S, Lokulo-Sodipe O, Horton R, Siebert R, Elbracht M, Temple IK, Eggermann T, Mackay DJG. Maternal variants in NLRP and other maternal effect proteins are associated with multilocus imprinting disturbance in offspring. J Med Genet. 2018 Jul;55(7):497-504. doi: 10.1136/jmedgenet-2017-105190. Epub 2018 Mar 24.

Appendix E Telephone prompt form

Study of Adults and Adolescents with Russell Silver syndrome in the UK (STAARS UK)

Checklist for telephone call with patients prior to research clinic visit will include:

1. **Discuss any queries that the family have about the study using details as written in information sheet**

2. **A prompt to families about various questions that are going to be asked at the interview**
   - i) Look up family history
   - ii) Look up drug history
   - iii) Remember age of puberty
   - iv) Write down known hospital admissions
   - v) Discuss if possible early feeding history
   - vi) Bring the 'Red book' if available to clinic
   - vii) Estimate or ask parents to find out their height and weight as adults at the time of the birth of the person with RSS
   - viii) Find out about the period around the pregnancy and delivery
   - ix) Ask RSS adults to find out about their childhood from a developmental and health perspective from a parent
   - x) Ask whether they mind being recorded and having photographs taken

3. **Find out the name of their GP and doctor who is currently or has previously provided most care with growth**

4. **Ask if parents can have a questionnaire sent to them if they are not coming to the clinic**

5. **Explain to the patient that the visit will involve questions about health and development and an examination.**

6. **Explain that some tests (DEXA scan and pQCT) will only be available at some research centres**

7. **Provide web address for study website and e-mail address**

8. **Give directions to the local centre and permission to request medical notes if they are stored at that centre**

9. **Explain that the participant needs to come to the clinic fasting (overnight or for at least 6 hours) and should not drink caffeine on the day of the appointment. For the 24 hours before, alcohol should not be drunk and no exercise performed.**

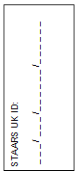
10. **Give participants the opportunity to withdraw from the study**

11. **Explain that everything will be discussed again and the option to withdraw will remain**

12. **If taking part, written consent will be requested at the study visit.**

REC reference number: 13/SC/0630
Version 1.1
Date: 08/11/13

Appendix F Doctor-managed record of history and examination with RSS proband


STAARS UK ID:
___/___/___/___/___

Study of Adults and Adolescents with Russell Silver syndrome in the UK (STAARS UK)

Doctor-managed record of history and examination with RSS proband (+/-parent/guardian depending on age)

Participant's full name:.....

Participant date of birth d d m m y y

Sex M = Male
F = Female

Post code

HISTORY AND EXAMINATION

Visit date d d m m y y

Location visit held

Parents and Conception

Indicate if information for this section on pregnancy and early history is gathered:

With proband and a parent present

From the proband alone

Maternal birth weight in kg Unknown

Paternal birth weight in kg Unknown

Maternal height at conception in cm and weight at conception in kg

Paternal height at conception in cm and weight at conception in kg

Did the participant's parents have any problem conceiving this child or other children?

History of infertility Yes No Unknown

Comments:

Did the participant's parents seek help with fertility?

Assistance with fertility in the pregnancy:

Yes No Unknown

If yes, what was used?

Was this child a monozygous twin?

Monozygous (identical) twin: Yes No Unknown

Did the participant's parents have any medical conditions requiring hospital treatment?

Diabetes in a father Yes No Unknown
Diabetes in a mother Yes No Unknown

Parental health issues comments:

Pregnancy

Was growth less than expected during the pregnancy?

Intrauterine growth retardation diagnosed in pregnancy Yes No Unknown

If yes, provide approximate stage in the pregnancy when it was first noted

Gestation 1st trimester 2nd trimester 3rd trimester

Tick any of the following noted in utero:

Bleeding
Polyhydramnios
Oligohydramnios
Smoking
Severe nausea and vomiting
Medication in pregnancy (including IVF drugs if applicable)

Drugs used

Folic acid taken in pregnancy Yes No Unknown

Dose, if known:

Did the participant's mother drink more than 4 units of alcohol per week during the pregnancy?

Yes No Unknown

Known infections

Occupational exposure (record unusual jobs)

Exposure comments

Birth

What was the method of delivery?

Type of delivery (tick)

Spontaneous
Emergency C-section
Elective C-section
Forceps
Other

Was the participant born late early or on time? Early Late Term

Gestation of pregnancy

Placenta: Normal Abnormal Unknown

Details:

At birth

Birth weight (g) .....

Birth length (cm) .....

Birth head circumference (cm) .....

Were there any unusual features at birth?

Congenital anomalies: Yes No Unknown

If yes, what?

Developmental

Were there worries about early development? ☐Yes ☐No ☐Unknown

Were there worries about reaching normal motor milestones? ☐Yes ☐No ☐Unknown

Were there any concerns about speech development? ☐Yes ☐No ☐Unknown

In more detail:

Age in months when
Sat unaided..... ☐Unknown
Crawled..... ☐Unknown
Walked unaided..... ☐Unknown
First said a few words..... ☐Unknown

Vision normal ☐Yes ☐No ☐Unknown
Hearing normal ☐Yes ☐No ☐Unknown
If no, provide details

Comments.....

.....

Schooling

Were there special educational needs at school? ☐Yes ☐No

In more detail

Nursery: mainstream ☐ special needs nursery ☐

Primary School: mainstream ☐ mainstream with special help ☐ special needs education ☐

Secondary school: mainstream ☐ mainstream with special help ☐ special needs education ☐

Indicate nature of special help.....

Statement of educational needs? ☐Yes ☐No ☐Unknown

Age of leaving education in years (if appropriate) .....

Were any of these qualifications achieved by the participant?

GCSEs ☐
O-levels ☐
CSEs ☐
A-levels ☐
Degree or other higher qualification ☐

Other educational qualifications:

.....

.....

.....

Family History

Pedigree to third generation relatives with date of births, first names, approximate heights, birth weights and significant illnesses warranting an appointment in hospital. Indicate who gave you the information.

Medical History

<u>Eating history</u>

Did the participant feed well as a newborn baby? ☐Yes ☐No ☐Unknown

Type of feeding and duration ☐ Breast-fed ..... ☐ Bottle-fed .....

Was tube feeding needed? ☐Yes ☐No ☐Unknown

If yes provide information including age when it improved

Was a gastrostomy ever needed? ☐Yes ☐No ☐Unknown

If yes provide information including age when it was reversed?

Appetite history – was the participant ever described as having a poor appetite?

☐Yes ☐No ☐Unknown

If yes, provide information and ages when it improved if it did:

.....

.....

.....

.....

.....

Direct questions

Have you got now or had in the past:

Joint hypermobility ☐Yes ☐No ☐Unknown

A history of having

episodes of hypoglycaemia ☐Yes ☐No ☐Unknown

episodes of excessive sweating ☐Yes ☐No ☐Unknown

Hypospadias repair (males) ☐Yes ☐No ☐Unknown

Cleft palate ☐Yes ☐No ☐Unknown

Abnormalities of hands and feet including incurving of little fingers ☐Yes ☐No ☐Unknown

Joint contractures ☐Yes ☐No ☐Unknown

Comments.....

.....

.....

<u>**General medical history in childhood, teenage years and adulthood**</u>

Have any medical conditions been diagnosed?

List conditions requiring attendance at hospital

Comments

Any known **height and weight measurements** at different ages recorded in hand held records

	Height (cm)	Weight (kg)
Age 1 Age 2		
Age 5 Age 10 Age 15		

Puberty

Girls
At what age did regular periods start? .....
At what age was pubic hair noticed? .....
At what age was breast development noticed? .....

Girls, age of puberty ☐

Boys
At what age was puberty thought to have started? .....
At what age was growth estimated to be complete? .....
Was puberty at the same time as friends at school? .....

Boys, age of puberty ☐

Medication required to prevent early puberty ☐Yes ☐No ☐Unknown
Medication required to initiate puberty ☐Yes ☐No ☐Unknown

Drug History

Growth Hormone (GH)

Was Growth Hormone taken? ☐Yes ☐No ☐Unknown

List age when started.....

Age when stopped.....

Dosages – where possible.....

Date	Dose	Dose per kg or m²	Last IGF level

First IGF-1 level..... After how long on treatment..... Dose.....

Peak IGF-1 level..... After how long on treatment..... Dose.....

Other medication

.....

List including ages when taken if known.....

Known allergies.....

Examination check list

Which hand does the participant write with?

1. Right
2. Left
3. Ambidextrous ☐

Head circumference in cm

. cm

. cm

. cm

Weight (preferably in light clothing such as shorts & t-shirt)

kg

kg

kg

Height in cm

. cm

. cm

. cm

Sitting height in cm

. cm

. cm

. cm

Left upper arm circumference (arm straight)

. cm

. cm

. cm

Right upper arm circumference (arm straight)

. cm

. cm

. cm

Left leg circumference (leg straight)

. cm

. cm

. cm

Right leg circumference (leg straight)

. cm

. cm

. cm

Hand size

Left (middle finger tip to wrist crease)

. cm

. cm

. cm

Hand size

Right (middle finger tip to wrist crease)

. cm

. cm

. cm

Waist circumference (standing)

. cm

. cm

. cm

Hip circumference maximum (standing)

. cm

. cm

. cm

Biceps skinfold

. mm

. mm

. mm

Triceps skinfold

. mm

. mm

. mm

Subscapular skinfold

. mm

. mm

. mm

Upper supra-iliac skinfold

. mm

. mm

. mm

Grip strength

	RIGHT SIDE	LEFT SIDE
GRIP STRENGTH (Record to nearest 0.5 kg)	□□ . □	□□ . □
	□□ . □	□□ . □
	□□ . □	□□ . □

FAT MASS ANALYSIS

	. kg
	. kg
	. kg

DEXA BONE MASS DENSITY

. g/m²

HRpQCT

Compact bone density..... mmg HA/ccm

Cortical thickness..... mm

Fat mass..... g

Morphology exam -

Face Overall gestalt; Silver Russell syndrome ☐Yes ☐No ☐Unknown

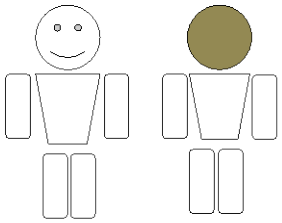
Dysmorphic features Familial .....
Non-familial.....

Triangular facies ☐Yes ☐No ☐Unknown

Skin Normal: ☐Yes ☐No ☐Unknown

Describe birth marks, neurocutaneous stigmata or cafe au lait spots
Pigmentary anomalies
Caferlaun, light skin, or unusual texture
Acanthosis nigricans
Plaques

Front Back



Body asymmetry? ☐Yes ☐No

Describe asymmetry

Cranium shape normal ☐Yes ☐No ☐Unknown

Describe skull shape if abnormal eg. brachycephaly

Ears Normal ☐Yes ☐No
Comments

Eyes Any unusual features noted

Nose Any unusual features noted

Philtrum Describe, e.g. long/short/smooth

Mouth

Tongue, normal size ☐Yes ☐No

Comment eg. asymmetry

Palate, normal ☐Yes ☐No

Gums normal ☐Yes ☐No

eg. thickened gums

Teeth normal ☐Yes ☐No

eg. sizeshapeforuption

Chin Normal ☐Yes ☐No

If no, is there micrognathia? ☐Yes ☐No

Comments

Hands and feet

Fingers

5th finger clinodactyly ☐Yes ☐No

Finger contractures ☐Yes ☐No

Comments e.g. which fingers are involved.....

Nails Normal examination ☐Yes ☐No

comments.....

Feet Normal examination ☐Yes ☐No

comments.....

Congenital anomaly identified ☐Yes ☐No

If yes, circle number of congenital anomalies: 1 2 3 4 5

Details

Chest Normal examination ☐Yes ☐No
Comments

Heart Normal examination ☐Yes ☐No

Comments

BP: Reading 1 Reading 2 Reading 3

Abdomen Normal examination ☐Yes ☐No

Comments

Back Normal examination ☐Yes ☐No

Comments

Nervous System Normal examination ☐Yes ☐No

Comments

Communication within normal spectrum ☐Yes ☐No

Indicate type of problem

Speech delay/limited	Mild	Moderate	Severe
Hearing deficit	Mild	Moderate	Severe
Visual problem	Mild	Moderate	Severe
Understanding	Mild	Moderate	Severe

Comments

Intellectual abilities within normal spectrum ☐Yes ☐No

Indicate type of development problem

Global delay:	Mild	Moderate	Severe

PUBERTAL ASSESSMENT for adolescents
(Tanner stages 1 to 5)

The pubertal assessment will be completed by the doctor. For any person preferring not to be examined a self-complete questionnaire will be provided.

Boys

Testicular volume (Orchidometer) ☐

Genital stage (Tanner stage) ☐

Pubic hair (Tanner stage) ☐

Girls

Breast development (Tanner stage) ☐

Pubic hair (Tanner stage) ☐

WELLBEING (questions)

Health

Would you say that your health is excellent, good, fair, or poor?
1. Excellent
2. Good
3. Fair
4. Poor ☐

School (if currently at school)/Job

How do you feel about school/job at present?
1. I like it a lot
2. I like it a bit
3. I don't like it very much
4. I don't like it at all ☐

Life satisfaction

Here is a picture of a ladder. The top of the ladder,10, is the best possible life for you and the bottom, 0, is the worst possible life for you. In general, where on the ladder do you feel you stand at the moment? Please point to the number that best describes where you stand.' ☐☐

Out of place

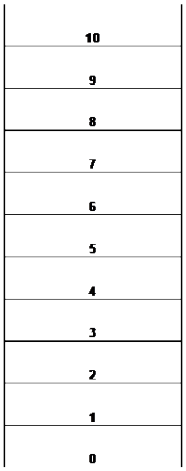
Here are some statements about your life. Please tell me if you agree or disagree with them:
1. Strongly agree
2. Agree
3. Disagree
4. Strongly disagree

I feel like an outsider or left out of things ☐

I feel awkward and out of place ☐

I feel lonely ☐

LADDER for question 9.3
(To be printed on a separate sheet and laminated)



Appendix G Response form

University Hospital Southampton NHS
NHS Foundation Trust

University of Southampton

Response form

Study of Adults and Adolescents with Russell Silver syndrome in the UK (STAARS UK)

I confirm that I have read the information sheet and I would like to be contacted by a member of the research team about arranging a study visit at a later date.

Please complete the details below:

Name .....

Signature .....

Date .....

Please provide some contact details:

Contact telephone number .....

Contact e-mail .....

Best time to contact you .....

Contact address.....

.....

Please return to:
Dr Kemi Sodipe
Wessex Clinical Genetics Service
Princess Anne Hospital
University Hospital Trust
Southampton SO16 5YA

Or put this form in the envelope provided and hand in to the researcher.

REC reference number: 13/SC/0630
Version 2
Date: 03/01/14

Appendix H Participant details form

Study of Adults and Adolescents with Russell Silver Syndrome in the UK (STAARS UK)

Participant Details

STAARS UK number (official site allocation): _ _ _ /_ _ _ /_ _ _ _ _ _ /_ _ _ _ _ _

(Site reference number/consecutive site numbers/participant date of birth (ddmmyy)/visit date; **added by primary research centre**)

Name of the patient:

Date of birth: _ _/_ _/_ _ Sex: ☐ Male ☐ Female

Address:

Postcode

Contact numbers:

Russell Silver subtype if known (tick):

1. ☐ H19 loss of methylation
2. ☐ Maternal UPD 7
3. ☐ Clinical diagnosis, test negative
4. ☐ Clinical diagnosis, no test performed
5. ☐ Other please comment.....

Contact Physician

Name:

Department & Hospital:

Contact number: E-mail:

General Practitioner

Name:

Address:

Contact number: E-mail:

REC reference number:13/SC/0630
Version 1
Date: 08/11/2013

Appendix I Outcome sheet

Study of Adults and Adolescents with Russell Silver Syndrome in the UK (STAARS UK)

Wessex Clinical Genetics Service
Princess Anne Hospital
University Hospital Southampton
Southampton SO16 5YA
Phone number: 02381 206551

STAARS UK ID: _/_/_ _ _ _ _ _ _ _/_

OUTCOME SHEET for ……

Height in cm

[ ][ ] . [ ] cm

Weight in kg

[ ][ ] . [ ] kg

Body mass index (BMI)

[ ][ ] . [ ] kg/m^2

Bone density

[ ][ ] . [ ] g/m^2

Blood pressure: / mmHg

Fasting blood sugar level……..……..

Cholesterol……..…..

Genetic result……..…….

Signature _____ Date _____

REC reference number: 13/SC/0630 Version 1 Date: 08/11/13

Appendix J Consent form for participating adult

Study of Adults and Adolescents with Russell Silver Syndrome in the UK (STAARS UK)

Wessex Clinical Genetics Service
Princess Anne Hospital
University Hospital Southampton
Southampton SO16 5YA
Phone number: 02381 206551

STAARS UK ID: _____/_____/_____/_____

CONSENT FORM

Participating adult

Please **initial** each box

1. I confirm that I have read and understand the information sheet dated __/__/__ about the above study. I have been given the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐
3. I understand that relevant sections of my medical notes and data collected during the study, may be looked at by the research team, by individuals from regulatory authorities, or from the _____ NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐
4. I give consent for my medical details to be used in published articles provided there is no link to my name. I understand that these may be available on the internet. ☐
5. I give consent to have a photograph taken for research purposes. ☐
6. I agree to blood and cheek samples being taken for DNA and biochemical analysis. ☐
7. **I agree to take part in the above study.** ☐

OPTIONAL:	Yes	No
8. I agree to a skin biopsy and/or hair bulb sample (circle to confirm).	☐	☐
9. I agree to have a DEXA scan and pQCT scan (where available).	☐	☐
10. I give consent for my photos to be used in published articles provided there is no link to my name. I understand that these may be available on the internet.	☐	☐
11. I would like to receive my cholesterol, fasting blood sugar test results and the diagnostic Russell Silver syndrome gene tests.	☐	☐
12. I would like to be told my blood pressure, height, weight, and body mass index. I would also like to be told my bone density if this test is available.	☐	☐
13. I agree that part of my sample may be sent to other collaborating researchers, even outside the UK, if only identification numbers are used.	☐	☐
14. I agree to my samples being used to make a cell line (more cells grown from my sample). I understand these may be used in future research related to growth or RSS and will not use my name.	☐	☐
15. I can be contacted about further studies related to this research project.	☐	☐
16. I give permission for DNA and the remainder of my blood sample (plasma) to be stored and used in other ethically approved studies related to growth or RSS, provided that the samples are linked anonymously.	☐	☐

Name of participant	Signature	Date
Name of person taking consent	Signature	Date

REC reference number: 13/SC/0630
Version 2 Date: 03/01/14

Appendix K Assent form

Study of Adults and Adolescents with Russell Silver Syndrome in the UK (STAARS UK)

Wessex Clinical Genetics Service
Princess Anne Hospital
University Hospital Southampton
Southampton SO16 5YA
Phone number 02381 206551

STAARS UK ID: __/__/__

ASSENT FORM FOR YOUNGER PARTICIPANTS

(to be completed by the young person with their parent/guardian's help if necessary)

Please tick each box if you agree (participant):

I have read the leaflet (or someone has read it to me).	☐
I understand why I've been invited to the clinic.	☐
I understand it's OK to stop at any time, if I want to.	☐
I agree to have a photo taken and understand that this might be used when the study results are reported.	☐
I agree to have a blood sample taken.	☐
I agree to have a picture taken of my bones if this is offered	☐
I am happy to take part in the study.	☐

Your name ______________________________

Date ______________________________

Your parent or guardian will have to fill in another form if they are happy for you to do the project.

REC reference number: 13/SC/0630
Version 2 Date: 03/01/14

Appendix L Consent form for participating relative

Study of Adults and Adolescents with Russell Silver Syndrome in the UK (STAARS UK)

Wessex Clinical Genetics Service
Princess Anne Hospital
University Hospital Southampton
Southampton SO16 5YA
Phone number: 02381 206551

STAARS UK ID: __/__/__/__

CONSENT FORM

Participating relative

Please **initial** each box

1. I confirm that I have read and understand the information sheet dated __/__/__ about the above study. I have been given the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐
3. I give consent for the research team to access my medical records if necessary, to obtain data relating to this study. I understand that this data will only be used for the purposes of this study, and may be anonymously reported in the study results when they are published (which may be online). ☐
4. I understand that relevant sections of my medical notes and data collected during the study, may be looked at by individuals from regulatory authorities or from _____ NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐
5. I agree to my saliva samples being taken for DNA analysis. ☐
6. **I agree to take part in the above study.** ☐

OPTIONAL:	Yes	No
7. I agree that part of my sample may be sent to other collaborating researchers, even outside the UK, provided it is not associated with any personal details.	☐	☐
8. I can be contacted about further studies related to this research project.	☐	☐
9. I give permission for DNA to be stored and used in other ethically approved studies related to RSS or growth, provided that the samples are linked anonymously.	☐	☐

Name of participant	Signature	Date
Name of person taking consent	Signature	Date

REC reference number: 13/SC/0630
Version 2 Date: 03/01/14

Appendix M Information booklet for participants or parents/guardians

Study of Adults and Adolescents with Russell Silver syndrome in the UK (STAARS UK)

INFORMATION BOOKLET

For participants and parents/guardians

Contact the research team to take part in this study

REC reference number: 13/SC/0630
Version 2 Date: 03/01/14

Part 1
Invitation

This is a study to find out more about what it means to have Russell Silver syndrome (RSS), a genetic condition affecting growth. We want to understand why it happens and whether there are long term health issues.

Any person aged 13 years or more who has been diagnosed with RSS can take part. If a diagnostic test for RSS has not yet been offered, we will help to arrange this.

Before deciding to take part, it is important to understand why the research is being done and what it will involve. Please take the time to read this carefully and discuss it with anyone you wish. Ask us if there is anything that is not clear or if you would like more information.

The study is trying to understand more about the development of people with Russell Silver syndrome as they get older. We are interested in growth, how the body is made up, and how the body uses energy (metabolism) in general. We are also finding out more about the major issues around living with Russell Silver syndrome. The study will look at the genetic cause and try to work out why it happened. One of the main reasons for the study happening now is that we are trying to find out whether the use of growth hormone has had an impact and so we want to include people who have and have not had this treatment. Participation in the study involves coming to a clinic at one of several hospital outpatient research centres across the UK for a discussion about medical history, an examination and some tests. The visit will last around 3 hours.

Taking part in this study is voluntary and does not impact on standard NHS care.

REC reference number: 13/SC/0630
Version 2 Date: 03/01/14

What are the possible benefits of taking part?

The main benefit of participating is that the study may help to improve the understanding of growth, development and health issues of people with Russell Silver syndrome, in the hope of improving knowledge so that appropriate treatment might be offered to people with this condition.

The study visit will be a one-to-one appointment with a clinician where a full health check will be performed over a longer time than is usually available at a healthcare appointment. By the end of the visit, any participant will hopefully have learnt more about his or her body and health, and discussed issues with a team who are meeting many people with this rare condition all the time. There will be the option to be given test results such as blood pressure and blood tests. If there are any problems identified with general health during the assessment, an appropriate referral can be made, with your permission.

Some people have been diagnosed with Russell Silver syndrome based on clinical assessment. They might not have been able to have a genetic test before or may have been tested when fewer genetic changes were known. Another benefit of this study is that those participants will be able to have the current tests for the known genetic changes.

What will happen at the research appointment and what measurements will be taken?

In brief

The study will involve talking to a research clinician, being examined, and having photos and a blood test taken. Because of the nature of the blood tests we need all participants to come to the clinic having fasted overnight (or for at least 6 hours). We will start with the blood sample followed by a snack before the rest of the interview.

In addition, we will ask everyone for permission to make contact about another part of the study, which involves a one-to-one interview about what it is like to have RSS.

REC reference number: 13/SC/0630
Version 2 Date: 03/01/14

Medical interview and examination

We will ask about medical, birth and family details, including information about early childhood, growth as a child, and any medication taken. There will be some questions about lifestyle using a short questionnaire.

If parents come to the study clinic, there is a questionnaire to complete; if not, we can post it where possible. This questionnaire asks about the conception, pregnancy, early medical problems, parent health and family health.

If agreed, we will perform a full medical examination including blood pressure and growth measurements such as height, weight, and head size. To check how well their lungs work, participants will be asked to blow into a lung machine. We will take measurements of skin thickness and grip strength, which tell us about muscle and fat. Passing a tiny electrical current between sensors stuck on the skin helps us work out body structure. This is tolerated well in many studies as it does not hurt and people only feel the stickers.

We will ask for permission for the research clinician to take photographs (of the face and full length, while standing fully clothed). These will help us to show how people with RSS look at different ages. The photos may be included when we write reports but only with agreement. It is important to let you know that some days reports are also available online so these photos would be on the internet but names would not be added.

Scans: if the study visit is at a centre where scans are available, we will measure the density of different bones. There are two different machines and they are painless. The DEXA scan measures bone density at the hip and spine whereas the pQCT scan measures the density in the arm and leg. To do both scans takes about 30 minutes. They have been used in many studies in children and adults before and are safe. They involve a very low dose of radiation – it is about the same as 3 days background radiation or the same as a chest x-ray. This will not have any implication for the participant's health and it's **optional.**

REC reference number: 13/SC/0630
Version 2 Date: 03/01/14

Blood: we would like to take a small sample of blood, about 2 tablespoons. We can use a cream or spray to numb the arm if wanted and then take a small sample with a needle. This procedure has been used for taking blood samples in many other studies and usually people cope well. The results will give us information on body chemicals and nutrients in the blood so that we can understand energy handling in the body.

Genetic testing: we would like to find out the genetic cause of Russell Silver syndrome. The test will involve taking a painless swab from the inside of the cheek. We will be looking for genes that might cause RSS using techniques that look at large parts of the genetic material. We may work with other scientists involved in RSS research. That could mean part of the sample being sent to other centres outside the UK. In that case the medical information would stay with the research team in Southampton and the sample would only be identified using the study number.

Hair and skin samples: at the study visit, we will ask participants aged 18 years and over if we may also take a hair sample and a skin sample. This will allow us to grow more cells in the laboratory – called a 'cell line' – and allow us to use the cells without having to request any further blood samples. We will be able to keep these cells growing indefinitely and use them for future research purposes. They may be used in ways we have not yet planned. These will not be labelled with a name. A participant does not need to have this test to join the study.

Examination, blood sample and genetics results

If something out of the ordinary is identified at the examination we will discuss it and, with permission, contact the appropriate GP if needed.

Most of the test results will not be provided as they will be performed together and so will be out of date by the time the results are known. But some results can be requested - like cholesterol, fasting blood sugar, body mass index, and (where available) bone density. Genetic diagnostic test results will be provided for participants on request or if they differ from diagnostic results already provided by the participant. The research is not designed to report any unexpected genetic findings unrelated to RSS.

We will send regular information updates on the overall results and progress of the study.

In depth interview
Participants will be asked if they would take part in another in depth discussion at another time, which could take around 2 hours. This is to try and discover more about what it means to live with Russell Silver syndrome. All participants will be asked if they would like to participate further, but only some people will actually be interviewed so we get a broad spread of ages.

Expenses and payments
Your travel expenses will be reimbursed at rates recommended by the University of Southampton.

What if there is a problem?
Any complaint about the study or any possible harm will be addressed. Detailed information on this is given in Part 2.

Is taking part in the study confidential?
As always, all information about participation in this study will be kept confidential. Details are included in Part 2.

Contact Details:
To contact the study team at any time phone:
02381 206551

This completes Part 1 of the Information Sheet. Please read Part 2 before making a decision.

Please turn over for Part 2

Questions

Part 2

What if there is a problem?
If you are worried about any aspect of this study, please speak to the researchers who will do their best to answer your questions.

Under our formal research procedures we are required to give you the following information:

If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure. Details can be obtained from the study coordinator. We are an experienced research team, and aim never to cause you harm. As outlined in Part 1, the planned investigations are considered safe. In the very unlikely event that something does go wrong and you/your child are harmed due to someone's negligence then you may have grounds for a legal action for compensation against the University Hospital Southampton but you may have to pay your legal costs.

Will our taking part in this study be kept confidential?
All information collected about you and your family during the course of the research will be kept strictly confidential. If we discover information that may be useful for your family doctor with your permission we will contact your doctor.

With your permission, the research team will have access to your medical notes. From time to time monitors and auditors from NHS Research and Development offices and regulatory inspectors may also require access to your clinical notes to check data from the study.

We will keep the information collected in the study for 15 years in line with research guidance for University Hospital Southampton. After this time it will be destroyed.

What will happen to the results of the research study?
The results of the study will be published in medical journals so that doctors and health professionals all over the world can understand what increases the likelihood of illness in child and adulthood with RSS. We

will also arrange for parent groups such as the Child Growth Foundation to write about the study results so that we can tell others what we have found.

We have a website that is kept updated with the findings from this study.
http://www.southampton.ac.uk/geneticsimprinting/information/patients/staars.page

Who is organising and funding the research?
This research is funded by the National Institute for Health Research (NIHR). The study is organised by the University Hospital Southampton and the University of Southampton.

Who has reviewed the study?
This study was given ethical approval by NRES Committee South Central – Hampshire B and the National Institute for Health Research.

THANK YOU FOR READING THIS BOOKLET

If you have any further questions, please feel free to ask

Contact us:

STAARS UK
Wessex Clinical Genetics Service
Princess Anne Hospital
Southampton
SO16 5YA
Tel: 02381 206551

REC reference number: 13/SC/0030
Version 2 Date: 03/01/14

To take part in this study either contact the health professional sending you this form or contact

The UK Russell Silver project team on

1. **Phone 02381 206551**
2. **E-mail: STAARS@suhs.nhs.uk**
3. **Return this section and send to**

Study of Adults and Adolescents with Russell Silver syndrome in the UK (STAARS UK)
Wessex Clinical Genetics Service
Princess Anne Hospital
Coxford Road
Southampton
SO16 5YA

Provide us with:

NAME

ADDRESS

PREFERRED CONTACT NUMBER

REC reference number: 13/SC/0030
Version 2 Date: 03/01/14

Appendix N Information leaflet for 13-15 year olds

Photos
We would like to take some photographs of you (fully clothed). These will help us show how people with RSS look at different ages. The photos may be included when we write reports if you agree. Reports are also available online so these photos would be on the internet but your name would not be added.

Do I have to take part?
No. It's up to you. If you agree to take part and then change your mind, you can stop at any time. It won't change how your doctors look after you.

Any questions?
If you have any questions, please ask your mum, dad or guardian. They can phone us if they need more information.

Study for Adults and Adolescents with Russell Silver Syndrome in the UK (STAARS UK)

Information Leaflet 13-15 years old

CONTACT DETAILS
Wessex Clinical Genetics Service
Princess Anne Hospital
Southampton
SO16 5YA

Tel number: 02381 206551

Ethics no: 13/SC/0630
Version 2
Date:03/01/14

What the study is about
(do ask for the adult study information sheet if you want more information)

We are doing a project to understand more about Russell Silver syndrome (RSS), which causes people to be shorter in height. We want to see how you are growing and developing, and find out more about you. This will involve some health checks.

Visiting our clinic
We will ask you not to have a meal for 6 hours (this might be overnight as normal when you are sleeping) before coming to see us so that we can measure your body's nutrients and test the genetics of RSS. The appointment is with a doctor who looks after children and young people.

Cheek swab & blood samples
We will get this over first. We rub the inside of your cheek with a swab, which is like a cotton bud. It does not hurt. Then we will ask you to let us take a small amount of your blood. We will put some cream on your arm first or use a spray. The cream and spray numb the skin so that you should not feel us take the blood with a needle. Once this is over you can have a snack.

The blood sample will be used to check your genetic make-up as well as other blood tests for sugar levels, fats and tests of how your kidneys and liver are working. We will do these to help us understand the results in RSS. We will let you and your parents know if we find problems. If there is anything your GP should do, we will ask your permission to contact him/her.

Talking
At our clinic we will talk and find out more about when you were born and if you have had any medical problems. The doctor will ask you lots of questions, for example about puberty and whether you take medicines.

Measurements
We will take measurements including your height, weight and skin thickness. We will also measure how hard you can squeeze with your hands and ask you to blow into a machine. To learn more about muscle and fat in your body we will pass a tiny pulse through one hand and one foot. All you will feel are the stickers. In some clinics, we can measure your body density. This is like having a picture taken and does not hurt. The first machine, called a 'DEXA' scan means you lie very still for a few minutes. The second machine is a type of scan where you put your arm or leg through a metal doughnut shaped machine and rest on the other side while the pictures are taken.

Checking over your body
The doctor will ask if we can examine you in the clinic and this will involve you taking off clothes to your underwear and letting the doctor listen to your heart and lungs and feel your abdomen. The doctor will be looking at whether puberty is complete as it tends to occur earlier in RSS. If you would prefer not to be examined for puberty, there wil be a sheet you can use to tell us which stage you think you are at.

Your general health
We will ask you to fill in a short questionnaire telling us about your family and how you feel.

Appendix O Information sheet for relatives

Study of Adults and Adolescents with Russell Silver syndrome in the UK (STAARS UK)

INFORMATION SHEET

For relative of a STAARS participant

Part 1
Invitation

This is a study to find out more about what it means to have Russell Silver Syndrome (RSS), a genetic condition affecting growth. We want to understand why it happens and whether there are long term health issues. We enclose a copy of the RSS study information sheet so that you understand more about why we are doing it.

A member of your family has decided to take part in this study and we are contacting you to see if you would

1. **complete a medical questionnaire and send it back**
2. **be willing to consent to your own medical records being examined if necessary** to find out recorded heights and weights and any health issues
3. **provide us with a saliva sample for genetic tests to find out more about the inheritance of RSS**

We ask for a sample of saliva to help us interpret the result in your relative. If you agree to take part in the study by returning the questionnaire, we will send you the required materials and instructions for taking the spit sample.

Genetics results
The results of any tests (or any other information or investigations) will be linked to your relative. We will not be sending results on your tests. We can send you regular information on the results and progress of the study if you would like them and you can contact us if there are issues you wish to discuss.

What are the possible benefits of taking part?
The main benefit is in knowing that you are taking part in a unique study that may help improve the understanding of growth, development and health issues of people with Russell Silver syndrome, in the hope of improving knowledge so appropriate treatment might be offered to people with this condition.

REC reference number: 13/SC/0630
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What if there is a problem?
Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. Detailed information on this is given in Part 2.

Will my taking part in the study be kept confidential?
As always, all information about your participation in this study will be kept confidential. Details are included in Part 2.

This completes Part 1 of the Information Sheet. If you wish to take part, please continue to read the extra information in Part 2 before making a decision.

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Version 2 Date: 03/01/14

REC reference number: 13/SC/0630
Version 2 Date: 03/01/14

Please turn over for part 2

Part 2

What if there is a problem?
If you are worried about any aspect of this study, please speak to the researchers who will do their best to answer your questions.

Under our formal research procedures we are required to give you the following information:

If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure. Details can be obtained from the study coordinator. We are an experienced research team and aim never to cause you harm. As outlined in Part 1, the planned investigations are considered safe. In the very unlikely event that something does go wrong and you are harmed due to someone's negligence then you may have grounds for a legal action for compensation against the University Hospital Southampton but you may have to pay your legal costs.

Will our taking part in this study be kept confidential?
All information collected about you and your family during the course of the research will be kept strictly confidential.

With your permission, the research team will have access to your medical notes. From time to time monitors and auditors from NHS Research and Development offices and regulatory inspectors may also require access to your clinical notes to check data from the study.

We will keep the information collected in the study for 15 years in line with research guidance for University Hospital Southampton. After this time it will be destroyed.

What will happen to the results of the research study?
The results of the study will be published in medical journals so that doctors and health professionals all over the world can understand what increases the likelihood of illness in child and adulthood with RSS. We will also arrange for parent groups such as the Child Growth Foundation

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to write about the study results so that we can tell others what we have found.

We have a website that is kept updated with the findings from this study: http://www.southampton.ac.uk/geneticimprinting/informationpatients/staars.page

Who is organising and funding the research?
This research is funded by the National Institute for Health Research (NIHR). The study is organised by the University Hospital Southampton and the University of Southampton.

Who has reviewed the study?
This study was given ethical approval by NRES Committee South Central – Hampshire B and the National Institute for Health Research.

THANK YOU FOR READING THIS BOOKLET

If you have any further questions, please feel free to ask

Contact us:

STAARS UK
Wessex Clinical Genetics Service
Princess Anne Hospital
Southampton
SO16 5YA
Tel: 02381 206551

REC reference number: 13/SC/0630
Version 2 Date: 03/01/14

Contact:

The UK Russell Silver project team on

1. Phone 02381 206551
2. E-mail: STAARS@uhs.nhs.uk

Study of Adults and Adolescents with Russell Silver syndrome in the UK (STAARS UK)
Wessex Clinical Genetics Service
Princess Anne Hospital
Coxford Road
Southampton
SO16 5YA

Appendix P Study protocol

Study of Adults and Adolescents with Russell Silver syndrome in the UK (STAARS UK)

Study Protocol

University Hospital Southampton and University of Southampton

A research project to find out more about what it means to have Russell Silver Syndrome (RSS), a genetic short stature syndrome and determine why it happens, how best to treat and whether there are long term unrecognised health issues.

Chief Investigator
Dr I Karen Temple
Prof of Medical Genetics
and Honorary Consultant in Clinical Genetics
Wessex Clinical Genetics Service
Princess Anne Hospital
University Hospital Southampton NHS Foundation Trust
Coxford Road
Southampton
e-mail: ikt@soton.ac.uk
tel: 023 8120 6166

Principal Investigator
Dr Kemi Lokulo Sodipe
Clinical Research Fellow
Wessex Clinical Genetics Service
Princess Anne Hospital
University Hospital Southampton NHS Foundation Trust
Coxford Road
Southampton
e-mail: dlsfe13@soton.ac.uk
tel: 023 8120 3995

Aim:

The primary aim of this retrospective cohort study is to provide urgently needed observational data on long term height and disease impact on adults with Russell Silver syndrome (RSS), so families (and health professionals) can be provided with more information when deciding how to optimally treat children with the condition.

Background

Russell Silver syndrome results in a significant restriction in final height (between 140 and 151 cm). It is usually due to a genetic fault on chromosome 11. At present children are offered Growth Hormone (GH) to improve adult height by about 4 cm but families do not know if there are any potential long-term risks with treatment. There are few studies on the outcome of the disorder in adults or whether GH makes a difference in this particular syndrome compared to other causes of short stature and low birth weight. GH may or may not have an impact on the risk for chronic long-term conditions like diabetes and obesity for patients with this condition.

A patient representative writes:
"Many RSS parents agonise over the Growth Hormone decision. Do you put your child through years of injections or do you potentially hold them back if as an adult they are at a disadvantage because of their height? What are the long-term risks of GH? Am I putting my child at risk? Maybe 20-30 years down the line, something will crop up that relates to treatment."

Not much is known about adult life with RSS or whether patients are particularly susceptible to early onset metabolic disease like diabetes or heart disease. The genes causing RSS are predicted to alter metabolism and GH may be more or less beneficial in this subgroup. It is also unknown whether certain genetic sub-groups are more susceptible or not.

This study, therefore, is to investigate patients with RSS from around the country, measuring height, quality of life, and factors which indicate metabolic disease like weight, blood pressure lipid levels, and blood sugar levels. We will then compare RSS patients with the same genetic cause who have been given GH with those who have not been given the treatment. We will conduct in-depth interviews with a subgroup of adults with RSS to identify important themes in people's lives. RSS is a rare disease and the prevalence in the UK is not known, so publicity will be as extensive as possible. The study hopes to recruit 100 people with RSS 13 years old and above.

Research Plan

1. Establish a cohort of patients with Russell Silver syndrome by recruiting patients from lay groups, national adverts through the lay groups and National Institute of Health Research (NIHR) Comprehensive Local Research Networks (CLRN) (Wellcome Clinical Research Facilities, Paediatric Endocrinology, Paediatrics, and Clinical Genetics)
2. Arrange a clinic visit at local participating centres for history and examination, blood and mouth swab sampling (and skin if possible if over 18 years) for metabolic testing and molecular subtyping
3. Report back to patients on specific clinical health check and gene diagnostic results
4. A subset of patients (30-34) will be asked to take part in a qualitative study to discuss living with Russell Silver syndrome in more detail
5. Correlate clinical, metabolic and genetic data
6. Transition to NHS service and patient information

Phase 1 – Data gathering

Inclusion criteria:

Patients diagnosed by a physician with Russell Silver syndrome and aged 13 years or above.

Protocol

A. Recruitment

Information about the study will be sent to the CLRN and paediatric, endocrine and genetics networks and the study advertised through these networks. Lay groups with RSS members will be alerted to the study and information about the study circulated as widely as possible including through a RSS lay website.

This is a rare condition with an unknown incidence and the main risk with the study is not getting enough cases.

Participants will be recruited in one of the five ways below:

1. Patients who have previously sought diagnosis for Russell Silver syndrome at Paediatric, Endocrine or Genetic clinics in NHS hospitals across the UK will be sent the patient information booklet and expression of interest form by their healthcare team.
2. New patients seeking diagnosis for Russell Silver syndrome attending Paediatric, Endocrine or Genetic clinics in NHS hospitals across the UK will be given the participant information booklet and expression of interest form by their healthcare team at the time of review in clinic. They would then indicate their permission to be contacted by the research team. Participants will be recruited via CLRN and the Wellcome Trust networks.
3. Regional genetics centres that routinely test for RSS, will be asked to send out study information to any referring doctors of patients with RSS, to be passed, if appropriate, to their patients.
4. Patients already part of the national imprinting study 'Imprinting Disorders Finding Out Why' who have indicated they are prepared to be re-contacted will be sent information. This is a study of patients – including some with RSS – that has been operational for the past 4 years and many people with RSS across the UK are already known to the researchers.
5. Self-referral from people with Russell Silver syndrome identified by national advertising and through lay groups.

Chase up letter

In order to improve uptake of the study, reminder letters will be sent out after 2 months to doctors known to look after people with RSS. Information will also be sent to CLRN networks at regular intervals.

B. Consent

NHS departments will have to decide whether they wish to be a recruiting centre with Research and Development approval OR signpost the patient to the Southampton RSS study centre for consent. Dual processes are needed as this is a very rare disease.

Once the patient has indicated that they are interested in taking part there are two options:

1. The local participant recruiting centre will discuss the study in detail using the telephone prompt form and information booklet then obtain consent from the participant or appropriate parent/guardian. The local recruiting centre will inform the Southampton study centre by e-mail – without any participant details – that they have recruited a participant. This will alert the Southampton research team to expect completed forms in the post. The local recruiting centre will keep the original consent form and send a copy of the consent form by post along with a completed participant detail form. When these are received, the Southampton research team will then contact the patient to confirm the study details and make an appointment for the patient to be seen at their local centre.
2. A potential participant may have been given information by a participant identification centre (and given his or her permission to be contacted) or may have expressed his or her interest directly to the Southampton research team. In these cases a member of the research team will telephone to discuss the study in detail. This conversation will involve the potential participant or relative being given details about the study and the clinic visit (including that they will be invited to the research clinic having not eaten for 6 hours) using the telephone prompt form and participant information booklet.

   During the telephone conversation, a suitable, convenient time for the research clinic, at the most local centre, will be agreed. Verbal consent to request the participant's medical records, if they are available at the visit site, will be taken over the phone. The participant or relative will be informed that full, written consent (by signing the consent form) will be obtained at the research clinic prior to the start of the interview and that medical notes will not be reviewed until this consent is confirmed. The researcher will explain that the participant or relative will still have the option not to take part.

The written consent will include access to medical records available at other hospitals, the taking of blood tests and photos, the use of imaging (DEXA and pQCT) in centres where available, and publication of the research results.

C. Study visit

Participants will be offered a 2-3 hour clinic appointment at their local genetics centre or research centre (agreement already in place from Clinical Research Facilities in Liverpool, Manchester, Newcastle, Sheffield, Southampton and Birmingham). Each participant will have a single unifying study number used for all samples and data. This participant number will be comprised of a three-digit site reference number, a three-digit number consecutively allocated to participants recruited at that site, the participant's six-digit date of birth, and the six-digit date of the study visit.

Participants will be asked to come to the appointment having fasted for 6 hours, and samples will be taken first followed by a snack for the participant.

1. A medical health questionnaire will be used to include past medical and family history, estimated parental heights, drug therapy and medical complications.
2. Medical examination will be performed, including dysmorphology review and i) blood pressure ii) waist circumference iii) hip circumference iv) growth parameters to estimate standard deviation scores (SDS) for height, weight, head circumference and Body Mass Index, v) limb symmetry vi) pubertal stage vii) skin pigmentation viii) where possible lung function.
3. Grip strength and total body fat mass will be measured. Fat mass will be measured using bio-impedance analysis, and where possible dual energy x-ray absorptiometry (DEXA) scan and peripheral quantitative computed tomography (pQCT).
4. Blood tests will be taken for: fasting triglyceride level, HDL/LDL cholesterol, glucose and insulin for HOMA-R, HbA1C levels, as well as thyroid function tests and 'C' peptide measurement, full blood count, liver and renal function tests, and IGF-1 levels. The tests for cholesterol, fasting sugar, full blood count, liver and renal function tests will be performed at the participating centre and results sent back to the Southampton study centre (associated with the participant name and ID number). The rest of the sample will be spun and frozen and stored at -80°C in the local centre (identified with a number alone) and transferred to one centre and tested in a batch for the remaining tests.
5. Blood and cheek samples to confirm genetic subtype will be requested (with a participant name and ID number).
6. Up to three assessments of wellbeing and Quality of Life questionnaires (QoLq) will be administered: i) wellbeing questions within the doctor managed record, ii) the SEIQoL-DW questionnaire and iii) the Sheehan disability scale to provide data on disability.
7. A skin biopsy and/or hair bulb samples will be requested from adult participants for cell line analysis so that further blood samples are not required to further investigate genetic subtype if warranted (this is optional).
8. Clinical photographs will be requested.
9. All participants will be asked whether or not they wish to be included in the group of participants offered an in-depth interview which will last around 2 hours. They will be informed however that only a subset of participants will be invited to take part in the qualitative arm of the study. This will use the phenomenological approach to explore the meaning of the condition in the lived experience of the patient. An interview will be conducted, where possible at a different time to the more medical component of the research and may occur at a person's home. From those who express an interest, 15-

17 people with RSS who have received GH and 15-17 people with RSS who have not received GH will be invited to interview. A purposeful sampling strategy will be adopted to ensure we interview across age and gender. Areas to be explored include: work and the impact of RSS, treatment decisions and experiences, friendships, schooling, intimate and parental relationships, independence, eating habits, community and identity issues. Interviews will be digitally sound recorded and later transcribed.

10. Previous medical records will be reviewed to confirm the Growth Hormone (GH) schedule and to enable gathering of anthropometric data at birth, at 2 years, pre-treatment or at 4 years, target height if predicted in clinic and evidence of any medical complications as reported by the patient (the notes will be requested from the hospital providing the main care for the GH treatment, with patient permission).

11. A questionnaire will be sent to parents who are potentially willing to provide information about their child's birth and early childhood (if they are not at the clinic, otherwise it will then be given directly). Both parents will also be sent an information sheet and a consent form for examination of medical records. After completed questionnaires and consent forms are received, parents will then be sent instructions and equipment for collection of a saliva sample to add information for the interpretation of genetic tests in their RSS relative.

12. Each participant will have a set of study research notes at Southampton University Hospital Trust. Correspondence will be recorded and the notes kept for 15 years in compliance with research governance for UHS.

Phase 2 – Data Analysis

Data will be entered onto a computer at the Wessex Clinical Genetics Service, University Hospital Southampton. A unifying identification number will be used for all databases. A separate database linking the identification number with the personal information will be created. These data will be used to make observations on the medical history, final height and markers of metabolic disease in the cohort (n =100) of adult patients with RSS confirmed by genetic investigation. Although for the majority they will have received treatment for many years, patients will be designated to the treatment-received group if they had GH for 12 months or more and the non-treated group if they received no GH or received it for less than 12 months.

Primary Outcomes

Statistical analysis will be undertaken to determine if there is a significant difference in mean final height in patients treated and not treated with GH. Height will be converted to age- and sex-adjusted SDS using current UK reference data. Treatment with GH will be used as a dichotomous binary indicator variable. T-tests and linear regression methods will be used for the analysis, the latter allowing for adjustment for confounding factors such as sex and age. Analysis will also be performed to explore any relationships between the metabolic parameters and GH and genetic subtype. A description of overall health issues and clinical findings will be made for the overall cohort.

A thematic analysis of the qualitative data will be undertaken to understand the lived experience of having Russell Silver syndrome as an adult or young person. Units of meaning will be identified and then grouped together as themes. A comparison will be made between the experiences of those who have been treated and those who have not. Where appropriate, illustrative vignettes/short cases will also be constructed from the data.

Phase 3 – Dissemination of results

Feedback to participants

There will be individual feedback given to patients verbally at the time of the examination during the clinic visit. Participants will be given the option of whether or not to receive results of blood tests for cholesterol and fasting blood sugar level. Participants who choose to will receive an outcome sheet with the results of their blood pressure, height, weight and BMI at the time of the study visit. Where DEXA and pQCT scanning are available, participants will also be able to choose to find out their bone density. For those who would like the blood test results in addition, these will be included on the outcome sheet and sent by post when results are received from the relevant centre.

Confirmation of the genetic diagnostic test by letter will be provided if patients opt to receive it. Any abnormality that may warrant referral for further investigation by the GP will be discussed with the participant and the GP contacted with the participant's permission.

Results of the study as a whole will be posted on the study website, published in medical journals and presented at lay group meetings. A study progress report will be produced yearly.

Appendix Q Sheehan Disability Scale

STAARS UK ID: __/__/__

Study of Adults and Adolescents with Russell Silver Syndrome in the UK (STAARS UK)

Sheehan Disability Scale (SDS)

Think about the effect that your symptoms had on your activities during the last week, and then circle one number for each question below:

1. How much did your symptoms disrupt your job, volunteer activities, or schoolwork?

Not at all	Mildly			Moderately			Markedly			Extremely
0	1	2	3	4	5	6	7	8	9	10

☐ I did not work, volunteer, or go to school during the last week for reasons other than illness.

2. How much did your symptoms disrupt your social life and leisure activities?

Not at all	Mildly			Moderately			Markedly			Extremely
0	1	2	3	4	5	6	7	8	9	10

3. How much did your symptoms disrupt your family life and home responsibilities?

Not at all	Mildly			Moderately			Markedly			Extremely
0	1	2	3	4	5	6	7	8	9	10

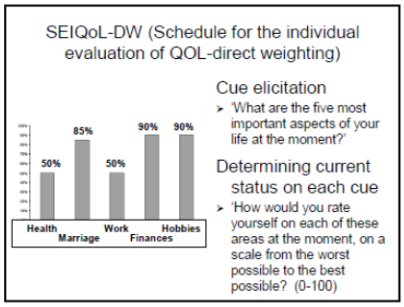
Please answer the following questions by placing a number from 0-7 in the blank on the right.

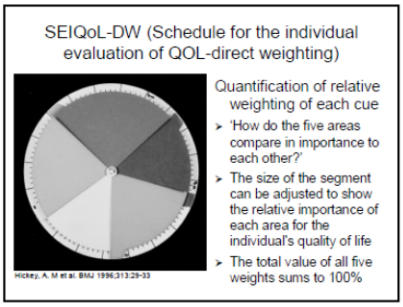
Days Lost
How many days in the last week did your symptoms cause your to miss work or school or make you unable to carry out your normal daily responsibilities at home? ______

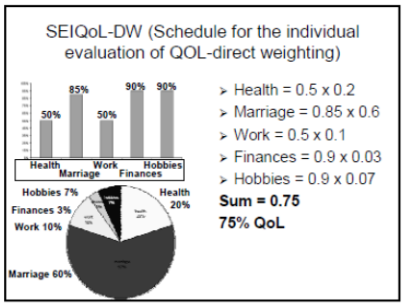
Days Unproductive
How many days in the last week did your symptoms cause you to be less productive, even though you went to work/school or attempted to carry out your normal daily responsibilities at home? ______

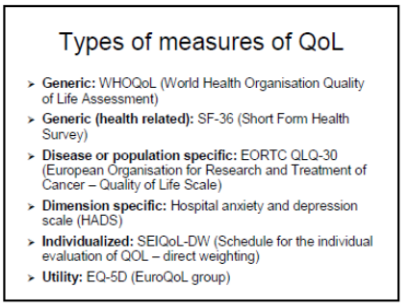
REC reference number: 13/SC/0630
Version 1
Date: 18/11/13

Appendix R Outline of Schedule for the Evaluation of Individual Quality of Life – Disability Weighted (SEIQoL-DW)









Appendix S Scale diagram for use with SEIQol-DW

Appendix T Female puberty questionnaire

Study for Adults and Adolescents with Russell Silver syndrome in the UK (STAARS UK)

STAARS UK ID: _/_/_____/_/

Wessex Clinical Genetics Service
Princess Anne Hospital
University Hospital Southampton
Southampton SO16 5YA
Phone number 02381 206551

Female puberty self-assessment questionnaire

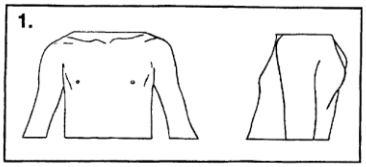
REC reference number: 13/SC/0630
Version 1 Date 08/11/13

We would like you to assess your chest development and body hair development

Section 1: Assessment of chest size

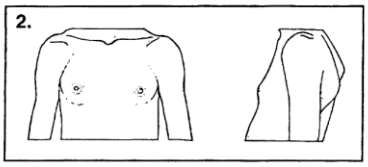
Please put a tick in the box that looks most like you now…

1.



The breasts are flat.

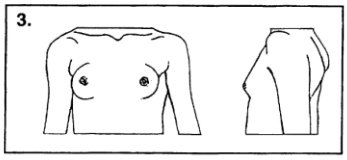
2.



The breasts form small mounds.

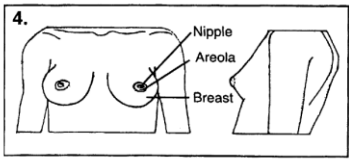
REC reference number: 13/SC/0630
Version 1 Date 08/11/13

3.



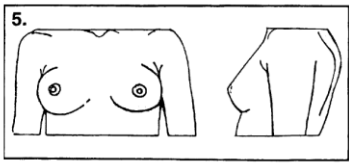
The breasts form larger mounds than in 2.

4.



The nipple and the surrounding part *(the Areola)* make up a mound that sticks up above the breast.

5.



Only the nipple sticks out beyond the breast.

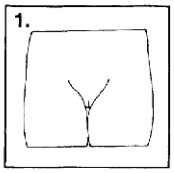
PLEASE TURN OVER……

REC reference number: 13/SC/0630
Version 1 Date 08/11/13

Section 2: Assessment of hair

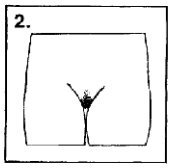
Please put a tick in the box that looks most like you now…

1.



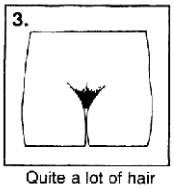
No hairs

2.



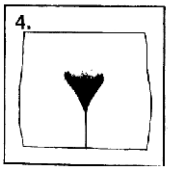
Very little hair

3.



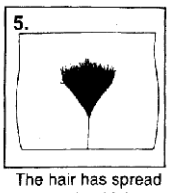
Quite a lot of hair

4.



The hair has not spread over the thighs

5.



The hair has spread over the thighs

THANK YOU FOR COMPLETING THIS QUESTIONNAIRE

REC reference number: 13/SC/0630
Version 1 Date 08/11/13

Appendix U Male puberty questionnaire

Study for Adults and Adolescents with Russell Silver syndrome in the UK (STAARS UK)

Wessex Clinical Genetics Service
Princess Anne Hospital
University Hospital Southampton
Southampton SO16 5YA
Phone number: 02381 206551

STAARS UK ID: _/_/_____/_____

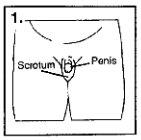
Male puberty self-assessment questionnaire

REC reference number: 13/SC/0630
Version 1 Date 08/11/13

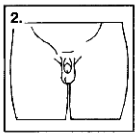
Please complete all 3 sections. We would like you to assess your penis growth, hair development and testicle size

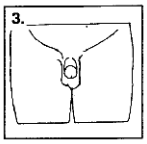
Section 1: Penis growth

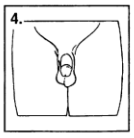
Please put a tick in the box that looks most like you now...

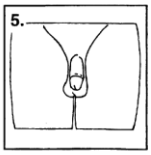
1. 

Scrotum and penis same size as when you were younger

2. 
The scrotum has lowered a bit and the penis is a little larger

3. 
The penis is longer and the scrotum is larger

4. 
The penis is longer and wider, the scrotum is darker and bigger than before

5. 
The penis and scrotum are the size and shape of an adult's

REC reference number: 13/SC/0630
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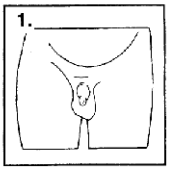
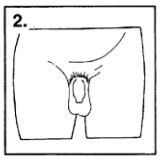
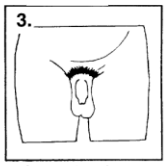
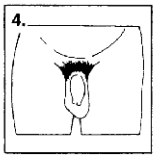
Section 2: Hair development

Please put a tick in the box that looks most like you now...

1. 
No hairs

2. 
Very little hair

3. 
Quite a lot of hair

4. 
The hair has not spread over the thighs

5. 
The hair has spread over the thighs

PLEASE TURN OVER.....

REC reference number: 13/SC/0630
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Section 3. Testicle development

Please look at the string of beads that the nurse has given to you.

Decide which size of bead most closely matches the size of your left and right testicles and write the number on the bead in each of the boxes below:

Left testicle bead size ☐

Right testicle bead size ☐

THANK YOU FOR COMPLETING THIS QUESTIONNAIRE

REC reference number: 13/SC/0630
Version 1 Date 08/11/13

Appendix V Questionnaire on early medical history for parent of STAARS participant

STAARS UK ID: _____/_____/_____/_____

Study of Adults and Adolescents with Russell Silver syndrome in the UK (STAARS UK)

Questionnaire on early medical history for parent of STAARS participant

REC reference number: 13/SC/0030
Version 1 Date:08/11/13

Name of person completing this questionnaire

_____

Relationship to study participant

_____

Information used to link data with study participant

Study participant's full name (person with RSS): _____

Study participant's date of birth: d d m m y y

Sex: M=Male F=Female

Postcode

_____

REC reference number: 13/SC/0030
Version 1 Date:08/11/13

To be completed by a mother/ father or guardian of a STAARS participant with Russell Silver syndrome.

The questions mainly relate to the early childhood of your son or daughter with Russell Silver syndrome but also ask a few questions about yourself and your partner. Please tick the answers you agree with. It is helpful to add comments.

1. Do you remember your and your partner's own birth weights?

Mother's **birth weight** ……(indicate pounds and ounces or kilos) Unknown ☐

Father's **birth weight**……(indicate pounds and ounces or kilos) Unknown ☐

Pregnancy

2. Do you remember your and your partner's height and weight just before the pregnancy started?

Mother's height (approximate) just before pregnancy…… (indicate feet and inches or cm) and weight……(indicate stone and pounds or kilos)

Father's height (approximate) just before the pregnancy …… (indicate feet and inches or cm) and weight…… (indicate stone and pounds or kilos)

3. a) Did you have any problem conceiving your child?

☐ Yes ☐ No ☐ Unknown

b) If yes, did you seek help from a fertility clinic?

☐ Yes ☐ No ☐ Unknown

c) Was Assisted Reproductive Technology used in the pregnancy?

☐ Yes ☐ No ☐ Unknown

If no, what other methods were used?

Comments, for example if you had problems in other pregnancies too:

4. a) Was your child a twin?

☐ Yes ☐ No ☐ Unknown

b) If yes were they identical?

☐ Yes ☐ No ☐ Unknown

REC reference number: 13/SC/0030
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The pregnancy continued…

5. a) Was the growth of your baby less than expected during the pregnancy?

☐ Yes ☐ No ☐ Unknown

b) If yes, provide approximate stage in the pregnancy when it was first noted:

Pregnancy stage: 0-12 weeks 13-26 weeks 27-40 weeks

6. Tick any of the following noted while your baby was in the womb:

Unexpected bleeding during the pregnancy ☐

Too much fluid around the baby (polyhydramnios) ☐

Too little fluid around the baby (oligohydramnios) ☐

7. Was folic acid taken in pregnancy? ☐ Yes ☐ No ☐ Unknown

Dose, if known……

8. Was any other medicine taken during the pregnancy?

☐ Yes ☐ No ☐ Unknown

Medicine(s) used

9. a) Did mother smoke during the pregnancy? ☐ Yes ☐ No ☐ Unknown

b) If yes, how many cigarettes a day?_____

10. Was severe vomiting a problem in the pregnancy? ☐ Yes ☐ No ☐ Unknown

11. a) Did mother drink alcohol during the pregnancy?

☐ Yes ☐ No ☐ Unknown

b) If yes, indicate how many units:

☐ <1 unit a week ☐ 1-4 units a week ☐ > 4 units a week

12. Were there any known infections during the pregnancy?

☐ Yes ☐ No ☐ Unknown

Specify if possible……

REC reference number: 13/SC/0030
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13. Did mother or father have an unusual job just before or during the pregnancy?

Exposure comments

Birth

14. What was the method of delivery? (please tick)

Spontaneous	☐
Elective C-section	☐
Emergency C-section	☐
Forceps	☐
Other	☐

15. a) Was your child born at the expected time? ☐ On time ☐ Early ☐ Late

b) If known, specify how many weeks of pregnancy (A full time pregnancy is 40 weeks)

16. Was the afterbirth (placenta) was described as:

☐ Normal ☐ Abnormal ☐ Unknown

Details:

At birth

17. Do you remember your child's:

Birth weight ..... (indicate pounds and ounces or kilos)

Birth length ..... (indicate pounds and ounces or kilos)

Birth head circumference, in cm .....

18. a) Was your child born with any unusual features at birth?

☐ Yes ☐ No ☐ Unknown

b) If yes, what?

Development

19. a) Were there worries about early development? ☐ Yes ☐ No ☐ Unknown

If yes:

b) Were there worries about reaching normal motor milestones?
☐ Yes ☐ No ☐ Unknown

c) Were there any concerns about speech development?
☐ Yes ☐ No ☐ Unknown

Any comments: .....

In more detail:

20. a) At what age in months did your child:

Sat unaided	☐ Unknown
Crawled	☐ Unknown
Walked unaided	☐ Unknown
First said a few words	☐ Unknown

b) Were there problems with eyesight? ☐ Yes ☐ No ☐ Unknown

c) Were there problems with hearing? ☐ Yes ☐ No ☐ Unknown

If yes, provide details:

Comments: .....

Schooling

21. Was your child ever described as having special educational needs at school?

☐ Yes ☐ No ☐ Unknown

22. What type of schooling did your child have?

Nursery: mainstream ☐ special needs nursery ☐

Primary school: mainstream ☐ mainstream with special help ☐ special needs education ☐

Secondary school mainstream ☐ mainstream with special help ☐ special needs education ☐

Indicate nature of special help if relevant: .....

23. Did your child have a statement of educational needs? ☐ Yes ☐ No ☐ Unknown

Medical History

Eating history

24. Did your child feed well as a newborn baby? ☐ Yes ☐ No ☐ Unknown

25. Did your child ever need to be fed via a tube? ☐ Yes ☐ No ☐ Unknown

If yes, provide information including age when it improved:

.....

26. Did your child ever need to be fed via a gastrostomy? ☐ Yes ☐ No ☐ Unknown

If yes, provide information including age when it was reversed?

.....

27. Did your child have prolonged poor appetite?

☐ Yes ☐ No ☐ Unknown

If yes, provide information and ages when it improved if it did:

.....

28. Did your child have excessive appetite?

☐ Yes ☐ No ☐ Unknown

If yes, at what age? .....

Direct questions

29. In your opinion did your child have:

Joint hyper-extensibility	☐ Yes	☐ No	☐ Unknown
A history of having			
- episodes of hypoglycaemia	☐ Yes	☐ No	☐ Unknown
- episodes of excessive sweating	☐ Yes	☐ No	☐ Unknown
Hypospadias repair (males)	☐ Yes	☐ No	☐ Unknown
Cleft palate	☐ Yes	☐ No	☐ Unknown
Abnormalities of hands and feet including incurving of little fingers	☐ Yes	☐ No	☐ Unknown
Bent joints (contractures)	☐ Yes	☐ No	☐ Unknown

Comments: .....

30. Do you have any record of your child's height and weight measurements at different ages recorded in hand held records?

	Height in cm	Weight in kg
Age 1		
Age 2		
Age 5		
Age 10		
Age 15		
Adult		

31. Do you remember when your child went into puberty?

Girls:
At what age did periods start? .....

Boys:
At what age do you estimate growth was complete? .....

32. Did your child have:

Medication given to stop early puberty	☐ Yes	☐ No	☐ Unknown
Medication given to start puberty	☐ Yes	☐ No	☐ Unknown

Drug History

Growth Hormone (GH)

33. Did your child ever take growth hormone? ☐ Yes ☐ No ☐ Unknown

What age was it started: .....

Age when stopped: .....

Dosages – where possible: .....

34. Did your child have other regular medication?

List including ages when taken if known: .....

Own Medical and Family History

34. Do you have any medical conditions requiring hospital treatment?

Mother: ☐Yes ☐ No ☐ Unknown

Father: ☐Yes ☐ No ☐ Unknown

Comments:.....

35. Please indicate if father or mother has diabetes :

Mother: ☐ Yes ☐ No ☐ Unknown

Father: ☐ Yes ☐ No ☐ Unknown

What type?.....

Age when diagnosed?.....

How are you treated?.....

36. Does anyone in the family have Russell Silver syndrome?

☐ Yes ☐ No ☐ Unknown

If yes, who? What is the relationship to your child?

.....

37. Does anyone have short stature? ☐ Yes ☐ No ☐ Unknown

If yes, please indicate approximate heights and the relationship to your child:

.....

38. Does anyone have excessive tall stature? ☐ Yes ☐ No ☐ Unknown

If yes, please indicate approximate heights and the relationship to your child:

.....

39. Is there any family history of medical conditions?

In particular:

i) Indicate conditions noted at birth.....

Also

ii) Indicate conditions requiring hospital appointments.....

If yes to these questions can you indicate the relationship to your child with RSS.....

.....

Further comments and space for any clarification

THANK YOU FOR COMPLETING THIS QUESTIONNAIRE

If you have any questions, please contact us:

Wessex Clinical Genetics Service
University Hospital Southampton
Southampton
SO16 5YA

Tel: 02381206551

Glossary of Terms

Café-au-lait macules	Areas/patches of skin pigmentation which are flat and pale brown ('coffee coloured') in colour

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