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Pallister-Killian Study

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ABSTRACT

FACULTY OF MEDICINE

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Doctor of Medicine

PALLISTER-KILLIAN STUDY

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Pallister-Killian syndrome is a rare condition, occurring in approximately 5 live births per million. It is a sporadic condition caused by mosaic tetrasomy of the short arm of chromosome 12 (12p). The main features are mental retardation, seizures, dysmorphic features and a variety of congenital malformations.

The results of a detailed study into Pallister-Killian syndrome in Great Britain are reported. Twenty-two patients with Pallister-Killian syndrome, ranging in age from four months to 31 years were recruited and comprehensive data on each of them obtained. The dysmorphic features, developmental abilities, clinical phenotype and natural history of the condition are described.

This study has identified a number of interesting features of the condition, including the surprising finding that 27.3% of the cohort only had mild or moderate mental retardation, the high frequency of anhydrosis or hypohydrosis, hyperventilation and breath holding episodes, and the tendency for seizures to be myoclonic. The dysmorphology was found to be more pronounced in those with more severe phenotypes but no evidence of a genotype-phenotype correlation was detected.

The difficulties in making the diagnosis in the absence of the classical features are discussed. Array CGH testing, which is rapidly becoming a first-line diagnostic test, was only able to detect the increased dosage of 12p in 16.7% of patients. All of these patients were aged less than four years, suggesting that the diagnostic yield of this testing in Pallister-Killian syndrome decreases in older patients. Although buccal FISH analysis was found to be positive in 75% of cases, it would only be performed in cases where the diagnosis was suspected. Clinical suspicion and skin biopsies will remain essential in making this diagnosis.

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Author's Declaration

I declare that this thesis is my own work. It is based mainly on my own original research work, with the work done by others acknowledged below. All published work consulted or quoted has been fully referenced and all sources of help are acknowledged below. It was done while in candidature for a research degree with this university and has not been submitted to another institution. None of this work has been published prior to submission of the thesis.

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All DNA analysis was performed in Wessex Regional Genetics Laboratory.
Microsatellite analysis was performed by Andrew Douglas, Academic Clinical Fellow, Wessex Clinical Genetics Service, under the supervision of Simon Thomas.
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FISH analysis was performed by Viv Maloney.

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Abbreviations Used In Text

ºC	Degrees Celsius
CGH	Comparative genomic hybridisation
Cy3	Cyanine 3 deoxyuridine triphosphate
Cy5	Cyanine 5 deoxyuridine triphosphate
DNA	Deoxyribonucleic acid
FISH	Fluorescence in situ hybridisation
i(12p)	Isochromosome 12p
IQ	Intelligence quotient
IUGR	Intra-uterine growth restriction
K_3-EDTA	Tripotassium ethylenediamine tetraacetic acid
kb	Kilobases
kDa	Kilodaltons
$\log_2$	2-base logarithm
Mb	Megabases
ml	Millilitres
MLPA	Multiplex ligation-dependent probe amplification
µg	Micrograms
µl	Microlitres
n/a	Not applicable
ng	Nanograms
nm	Nanometres
NHS	National Health Service
OMIM	Online Mendelian Inheritance in Man
p	Probability
PCR	Polymerase chain reaction
R&D	Research and development
SPSS	Statistical Package for the Social Sciences

Abbreviations Used In Tables and Figures

+	Feature present
-	Absence of feature recorded in report
CI	Confidence interval
D	Day (of life)
F	Female
$\log_e$	Natural logarithm
M	Male
mo	Months (age)
n	Number
PKS	Pallister-Killian syndrome
RR	Relative risk
SE	Standard error
y	Years (age)

1: INTRODUCTION

Pallister-Killian syndrome is a rare condition, with an estimated prevalence of less than 1 per 10,000 births[1]. This is a sporadic condition and no reports of familial recurrence have been found. It is caused by mosaic tetrasomy of the short arm of chromosome 12 (12p) and is sometimes referred to as 'mosaic tetrasomy 12p' or 'tetrasomy 12p'. The condition was first described in adults by Pallister et al[2] in 1977. Teschler-Nicola and Killian[3] later reported a child with a phenotype similar to that subsequently reported in children with mosaic tetrasomy 12p. This led to the name 'Pallister-Killian syndrome'.

Patients with Pallister-Killian syndrome have dysmorphic features, which vary with age. Severe mental retardation is the norm and seizures are very common.

A full literature review, described in 3a: Literature Review was performed and the details of this are included in the remainder of this introductory text and the associated summary table (Table 4). Full details of the phenotype of each reported live-born case are included in Table 21 to Table 27 in 7a: Appendix 1.

1a: Phenotype

Polyhydramnios is common in pregnancies where the fetus has Pallister-Killian syndrome. Increased nuchal translucency, both with[4;5] and without[6-9] cardiac defects and intra-cranial ventriculomegaly[9-12] have also been reported during the antenatal period. Other congenital anomalies that form part of this syndrome, such as diaphragmatic hernia and limb abnormalities may also be detected on antenatal ultrasound scanning.

The majority of the reported patients have had a normal or relatively high birth weight, although intra-uterine growth retardation has occasionally been reported[6;7;13;14]. Macrocephaly is also common at birth, although microcephaly has been reported once[15]. Birth length is usually within normal limits but there are some reports of increased length[16-21] and one of low birth length[22]. Hypotonia is usually present from birth and may cause feeding difficulties and failure to thrive.

The dysmorphic features of Pallister-Killian syndrome include hypertelorism, epicanthic folds, flat nasal bridge, anteverted nares, long philtrum, large mouth with a cupid's bow appearance, low-set and posteriorly rotated ears and clinodactyly. In young children, a prominent forehead with high anterior hairline, sparse eyebrows and eyelashes and areas of alopecia are the norm but these improve with age. Micrognathia may also be noted in younger children but prognathia is more common in older children. The face also tends to become coarser as the child ages. The dysmorphic features present in live-born individuals with this condition are shown in Table 22 (page 185).

Almost all patients known to have Pallister-Killian syndrome have severe mental retardation, with the majority never learning to walk or talk. Only four patients with milder phenotypes have been reported[18;20;23;24]. One of these patients had a formal assessment at the age of 13 years, with a full-scale IQ level of 93[24]. No IQ assessments are reported on the others but one coped in mainstream schooling until he was 14 years old[18] and another was coping in mainstream schooling at the age of seven years[20]. There is less detail on the final patient, who had delayed gross motor development

recorded at three years of age and an uneven pattern of developmental skills recorded at five years of age, with communication being the main problem at that stage[23].

Seizures are frequently reported, commencing any time from the neonatal period[25-27] through to adulthood[28]. The type of seizure is often omitted from case reports but, where it is recorded, generalised tonic-clonic seizures are most common. Both myoclonic seizures[21;29] and late-onset West syndrome, with[30] or without[8;21] hypsarrhythmia have been recorded. Table 21 (page 180) summarises the neurological features seen in the reported live-born individuals with Pallister-Killian syndrome.

A number of intra-cranial malformations have been described in individual case reports, as shown in Table 1. The most common are neuronal migration defects[10;29;31;32] and abnormalities of myelination[8;33;34]. Dandy-Walker malformation[35], atrophy of the corpus callosum[27] and cerebellar hypoplasia[36] have been described in individual patients. Cerebral atrophy and ventricular dilation are frequently reported but hydrocephalus requiring shunt insertion is a rare occurrence[29;37].

Table 1: Intra-cranial anomalies reported with Pallister-Killian syndrome

Reported in 5 or more patients
Polymicrogyria[10;31;32;38;39]
Heterotopia[10], unspecified myelination defect[25;29;33;34] or delayed myelination[8;31]
Reported in 2 patients
Calcification[29;32]
Hydrocephalus[29;37]
Peri-ventricular leucomalacia[20;27] but one was premature baby (28 weeks)[20]
Reported in single patient
Basal ganglia and pontine lesions– possibly ischaemic[25]
Atrophy of the corpus callosum[27]
Cerebellar hypoplasia[36]
Dandy-Walker malformation[35]
Olfactory bulbs and tracts absent[40]
Pineal gland enlarged[27]
Pineal tumour detected at 15[41]
Putamen and parietal lesions– similar to those seen in neurodegenerative disorders[25]

Diaphragmatic, inguinal and umbilical hernias are frequently seen in this condition. Exomphalos has also been reported in a few cases[10;33;42-44]. A wide variety of

congenital cardiac defects have also been described. These include ventricular septal defect[21;45;46], atrial septal defect[12;31;47-50], hypoplastic left heart[4], tetralogy of Fallot[35;51], Ebstein's anomaly[5], cardiomegaly[52], progressive right ventricular hypertrophy[26], hypertrophic cardiomyopathy[53;54], pericardial agenesis[55] and a variety of valvular abnormalities[12;27;46;47;56-58], as shown in Table 2. Lymphoedema[12;40;47;49;51;59] has also been described in a few patients.

Table 2: Cardiac anomalies reported with Pallister-Killian syndrome

Septal defects
Atrial septal defect (6 patients)[12;31;47-50]
Patent foramen ovale[43;58]
Ventricular septal defect (2 patients)[21;45;46]
Valvular anomalies
Aortic stenosis[57]
Bicuspid aortic valve with stenosis[47]
Mitral insufficiency (2 patients)[56;58]
Mitral and tricuspid insufficiency (2 patients)[12;46]
Tricuspid insufficiency[27]
Complex congenital heart disease
Ebstein's anomaly[5]
Hypoplastic left heart[4]
Tetralogy of Fallot[35;51]
Hypertrophy
Hypertrophic cardiomyopathy[53;54]
Right ventricular hypertrophy[26;29] or right-sided hypertrophy[60]
Other
Cardiomegaly[52]
Dysrhythmia (2 patients)[2]
Left ventricle "abnormal"[32]
Patent ductus arteriosus (10 patients, although one was premature)[12;20;21;32;50;54;61;62]
Pericardial agenesis[55] or defect[43]

Accessory spleens have been described[14;50;52;60] and in some cases, numerous small spleens are present[6;63]. Both choanal[57] and oesophageal[64] atresia has been reported. Anal atresia[10;12;31;32;43;44;59;62;65-68] has been found in a number of individuals with Pallister-Killian syndrome. The absence of the ovaries[40] and presence of a bicornate uterus[36] and breast asymmetry[2] have all been recorded in females. Hypospadias has been reported in males with Pallister-Killian syndrome[52], as has immaturity of the

secondary sexual characteristics[2]. Supernumerary nipples appear to be a common feature in both sexes.

Short stature and rhizomelic limb shortening are mentioned in many case reports, as are short hands and feet and hypoplastic nails[10;13;26;29;46;52]. Unilateral single palmar creases seem to be a common finding. A number of other skeletal abnormalities have been reported less frequently. These include polydactyly of the fingers[36;37;53;69-71], triphalangeal thumbs[31], pre-axial polydactyly of the foot/ hallux duplication[5;36;37;46;50;62;70], post-axial polydactyly of the foot[14;69], the presence of only 11 pairs of ribs[10;12;42;48;50] (or in one case only 10 pairs of ribs[44]), delayed bone age[2;3;5;10;29;41;72], hypoplastic vertebral bodies[29], atlanto-occipital fusion[5;26], congenital dislocation of the radial heads[45] and the presence of a sacral appendage[12;22;59;73]. The skeletal features reported in live-born individuals with Pallister-Killian syndrome are summarised in Table 23 (page 190).

Both hypopigmented and hyperpigmented lesions are seen in this condition, often in the same patient. These are usually discrete patches of altered pigmentation but, in a small number of cases, the pigmentary abnormalities have followed Blaschko's lines[19;21;74;75]. The hypopigmented lesions may only be visible with ultra-violet light[76]. Retinal pigmentary mosaicism has also been documented[61], as has retinal pallor [31;77-79].

In a small number of cases, corneal clouding has been described in infancy[40;57;80] but the cause of this was not established and each of these babies died shortly after birth. Colobomas[57;61], microphthalmia[66] and anophthalmia[10] have also been described.

Sensorineural hearing impairment has been reported in some cases[21;23;27;31;45;47;70;75;79;81-86] and may have been missed in others. Posterior auricular pits have also been reported in a single patient[75]. The palate is often highly arched or cleft and cleft uvula[45;57;65;70;84;87;88] is a relatively common occurrence. Cleft lip, on the other hand, has only been described twice[56;66]. Delayed eruption of the teeth has also been mentioned in a number of reports[3;29;45-47;84].

Table 3: Infrequent findings in Pallister-Killian syndrome

Reported in 3 patients
Sacral appendage[12;22;59;73]
Corneal clouding in infancy[40;57;80]
Reported in 2 patients
Cataracts[2]
Coloboma[57;61]
Iris dilator atrophy; nystagmus[20;79]
Atlanto-occipital fusion[5;26]
Reported in single patient
Microphthalmia[66]
Anophthalmia[10]
Optic atrophy[37]
Choanal atresia[57]
Posterior auricular pits[75]
Severe laryngomalacia requiring tracheostomy[62]
Oesophageal atresia[64]
Breast asymmetry[2]
Absent ovaries[40]
Bicornate uterus[36]
Focal cutis aplasia[55]
Hemihypertrophy[23]
Congenital dislocation of radial head[45]
Radio-ulnar synostosis[65]
Triphalangeal thumbs[31]
Butterfly vertebrae[12]
Hypoplasia of cervical vertebral bodies (C3-5)[29]
Fore-shortened vertebral bodies[2]
Increased height of lumbar vertebral bodies[3;84]
Inferior beaking and columnisation of lumbar vertebrae[89]
Coccygeal teratoma; spina bifida[84]
Sacral pits[75]
Immature secondary sexual characteristics[2]

Natural History

Most reported cases are young children, with only 5 adolescents (aged 12- 17 years)[18;24;37;41;74] and 8 adults[25;28;37;47;84] reported in the medical literature, which makes it difficult to obtain detailed information on the adult phenotype. In general, the face becomes coarser with age and macroglossia becomes more apparent. Cataracts have been reported in two patients aged 19 and 37 years[2] and optic atrophy described in one

adult[37]. In one case a pineal tumour was detected in one patient at the age of 15 years[41] but no other reports of tumour formation have been found.

Most of the adult patients reported have developed hypertonia, contractures and scoliosis[2;25;37]. Interestingly, both of the patients reported by Saito et al[25] had lost motor skills during adulthood. There is no documentation of regression in the other available reports and it is unclear whether this represents developmental regression or simply progression of their neuro-muscular difficulties. Two of the adults reported by Reynolds et al[47] were described as being ambulatory, which makes regression less likely.

There is anecdotal evidence that many patients with Pallister-Killian syndrome die at relatively young ages and the paucity of reports describing the adult phenotype would support this. Although many of the reports in the literature concern termination of pregnancy following antenatal diagnosis or neonates who have died due to congenital malformations, there are many reports describing the phenotypes of children with Pallister-Killian syndrome in the late 1980s and early 1990s and their further progress has not been reported. Two reports describe children who died at 20 and 33 months of age[47;53] and one records that the patient died at the age of 10 years[84] but the longevity of patients with Pallister-Killian syndrome has not been researched systematically. The oldest case reported in the literature was 45 years of age[47].

Table 4: Summary of features seen in reported cases

	Confirmed i(12p)					i(12p) not confirmed	Atypical karyotype
	Reported as neonates (n=30)		Reported when older (n=74)		Mathieu[52] paper		
	Present*	Absent*	Present*	Absent*	(n=10)	(n=8)	(n=9)
General and neurological							
Polyhydramnios	20 (66.7%)	0	11 (14.9%)	0		1 (12.5%)	3 (33.3%)
Birth weight: high	6 (20.0%)	18 (60.0%)	10 (13.5%)	49 (66.2%)		1 (12.5%)	2 (22.2%)
Birth weight: IUGR	2 (6.7%)	22 (73.3%)	0	59 (79.7%)		0	1 (11.1%)
Macrocephaly at birth	5 (16.7%)	9 (30.0%)	3 (4.1%)	25 (33.8%)		1 (12.5%)	1 (11.1%)
Microcephaly at birth	0	14 (46.7%)	0	28 (37.8%)		0	2 (22.2%)
Birth length: high	0	13 (43.3%)	6 (8.1%)	35 (47.3%)		1 (12.5%)	0
Birth length: low	0	13 (43.3%)	1 (1.4%)	40 (54.1%)		0	1 (11.1%)
Feeding difficulties	2 (6.7%)	0	12 (16.2%)	2 (2.7%)		5 (62.5%)	0
Failure to thrive	0	0	6 (8.1%)	6 (8.1%)		0	3 (33.3%)
Hypotonia as infant	3 (10.0%)	0	54 (73.0%)	1 (1.4%)	10 (100%)	8 (100%)	6 (66.7%)
Later hypertonia	n/a	n/a	6 (8.1%)	0		0	0
Mental retardation	n/a	n/a	65 (87.8%)	0	10 (100%)	8 (100%)	8 (88.9%)
-profound	n/a	n/a	11 (14.9%)	0	10 (100%)	0	0
-severe	n/a	n/a	13 (17.6%)	0		6 (75.0%)	0
-mild	n/a	n/a	4 (5.4%)	0		0	1 (11.1%)
Absent speech	n/a	n/a	17 (23.0%)	5 (6.8%)		3 (37.5%)	3 (33.3%)
Seizures	1 (3.3%)	0	39 (52.7%)	7 (9.5%)	7 (70.0%)	2 (25.0%)	2 (22.2%)
Macrocephaly	n/a	n/a	7 (9.5%)	46 (62.2%)		0	1 (11.1%)
Microcephaly	n/a	n/a	9 (12.2%)	44 (59.5%)		1 (12.5%)	2 (22.2%)
Ventriculomegaly/ atrophy	8 (26.7%)	4 (13.3%)	30 (40.5%)	10 (13.5%)	3 (30.0%)	2 (25.0%)	2 (22.2%)

	Confirmed i(12p)					i(12p) not confirmed	Atypical karyotype
	Reported as neonates (n=30)		Reported when older (n=74)		Mathieu[52] paper		
	Present*	Absent*	Present*	Absent*	(n=10)	(n=8)	(n=9)
Other intracranial abnormalities	7 (23.3%)	3 (10.0%)	10 (13.5%)	16 (21.6%)		1 (12.5%)	1 (11.1%)
Hearing impairment	0	0	17 (23.0%)	4 (5.4%)		4 (50.0%)	3 (33.3%)
Visual impairment	0	0	6 (8.1%)	0	9 (90.0%)	1 (12.5%)	0
Nystagmus	0	0	6 (8.1%)	1 (1.4%)		2 (25.0%)	3 (33.3%)
Optic nerve atrophy/ hypoplasia	0	0	3 (4.1%)	4 (5.4%)		0	0
Retinal pallor	0	0	3 (4.1%)	3 (4.1%)		0	1 (11.1%)
Hypopigmented lesions	1 (3.3%)	1 (3.3%)	40 (54.1%)	8 (10.8%)	3 (30.0%)	3 (37.5%)	3 (33.3%)
Hyperpigmented lesions	0	1 (3.3%)	22 (29.7%)	7 (9.5%)	1 (10.0%)	1 (12.5%)	0
Lymphoedema	3 (10.0%)	0	8 (10.8%)	1 (1.4%)		0	0
Redundant/ lax skin	12 (40.0%)	3 (10.0%)	14 (18.9%)	2 (2.7%)		3 (37.5%)	1 (11.1%)
Areas of hypertrichosis	1 (3.3%)	0	4 (5.4%)	0		0	0
Facial features							
Prominent forehead	8 (26.7%)	0	58 (78.4%)	0		5 (62.5%)	8 (88.9%)
Sparse hair/ areas of alopecia	10 (33.3%)	1 (3.3%)	59 (79.7%)	1 (1.4%)		8 (100%)	9 (100%)
Sparse eyebrows	3 (10.0%)	0	33 (44.6%)	3 (4.1%)		4 (50.0%)	2 (22.2%)
Synophrys	0	3 (10.0%)	4 (5.4%)	33 (44.6%)		0	0
Sparse eyelashes	1 (3.3%)	2 (6.7%)	7 (9.5%)	3 (4.1%)		5 (62.5%)	0
Hypertelorism/ telecanthus	19 (63.3%)	1 (3.3%)	52 (70.3%)	2 (2.7%)		6 (75.0%)	5 (55.6%)
Up-slanting palpebral fissures	3 (10.0%)	2 (6.7%)	20 (27.0%)	13 (17.6%)		3 (37.5%)	2 (22.2%)
Short palpebral fissures	5 (16.7%)	0	9 (12.2%)	0		1 (12.5%)	1 (11.1%)
Epicanthic folds	5 (16.7%)	0	32 (43.2%)	5 (6.8%)		3 (37.5%)	2 (22.2%)
Ptosis	0	0	10 (13.5%)	1 (1.4%)		6 (75.0%)	2 (22.2%)

	Confirmed i(12p)					i(12p) not confirmed	Atypical karyotype
	Reported as neonates (n=30)		Reported when older (n=74)		Mathieu[52] paper		
	Present*	Absent*	Present*	Absent*	(n=10)	(n=8)	(n=9)
Cataracts	0	3 (10.0%)	3 (4.1%)	6 (8.1%)		0	0
Corneal clouding	3 (10.0%)	0	1 (1.4%)	8 (10.8%)		0	0
Flat nasal bridge	11 (36.7%)	0	34 (45.9%)	1 (1.4%)		6 (75.0%)	4 (44.4%)
Broad nasal bridge	9 (30.0%)	0	30 (40.5%)	2 (2.7%)		5 (62.5%)	3 (33.3%)
Short nose	10 (33.3%)	0	24 (32.4%)	2 (2.7%)		6 (75.0%)	5 (55.6%)
Anteverted nares	11 (36.7%)	0	38 (51.4%)	2 (2.7%)		6 (75.0%)	4 (44.4%)
Full cheeks	1 (3.3%)	0	20 (27.0%)	1 (1.4%)		2 (25.0%)	2 (22.2%)
Long philtrum	8 (26.7%)	1 (3.3%)	31 (41.9%)	5 (6.8%)		6 (75.0%)	7 (77.8%)
Macrostomia	3 (10.0%)	0	24 (32.4%)	1 (1.4%)		2 (25.0%)	4 (44.4%)
Cupid’s bow appearance to mouth	2 (6.7%)	0	15 (20.3%)	0		5 (62.5%)	1 (11.1%)
Cleft lip	1 (3.3%)	13 (43.3%)	0	24 (32.4%)		0	0
Cleft palate	5 (16.7%)	9 (30.0%)	5 (6.8%)	19 (25.7%)		0	2 (22.2%)
Cleft uvula	1 (3.3%)	0	5 (6.8%)	0		1 (12.5%)	0
High arched palate	10 (33.3%)	0	19 (25.7%)	3 (4.1%)		6 (75.0%)	3 (33.3%)
Macroglossia	2 (6.7%)	0	29 (39.2%)	3 (4.1%)	2 (20.0%)	1 (12.5%)	2 (22.2%)
Micrognathia	5 (16.7%)	0	8 (10.8%)	15 (20.3%)		4 (50.0%)	2 (22.2%)
Prognathia	0	5 (16.7%)	13 (17.6%)	10 (13.5%)		0	0
Low-set ears	17 (56.7%)	0	27 (36.5%)	5 (6.8%)		3 (37.5%)	5 (55.6%)
Posteriorly rotated ears	7 (23.3%)	0	13 (17.6%)	5 (6.8%)		0	2 (22.2%)
Small ears	10 (33.3%)	0	11 (14.9%)	7 (9.5%)		0	2 (22.2%)
Large ears	0	10 (33.3%)	3 (4.1%)	15 (20.3%)		0	2 (22.2%)
Coarse face	8 (26.7%)	0	30 (40.5%)	2 (2.7%)		2 (25.0%)	2 (22.2%)

	Confirmed i(12p)					i(12p) not confirmed	Atypical karyotype
	Reported as neonates (n=30)		Reported when older (n=74)		Mathieu[52] paper		
	Present*	Absent*	Present*	Absent*	(n=10)	(n=8)	(n=9)
Skeleton and limbs							
Short stature	n/a	0	20 (27.0%)	21 (28.4%)		0	2 (22.2%)
Craniosynostosis	0	0	3 (4.1%)	2 (2.7%)		0	0
Large fontanelles/ delayed closure	2 (6.7%)	0	12 (16.2%)	2 (2.7%)	10 (100%)	1 (12.5%)	1 (11.1%)
Delayed eruption of teeth	n/a	0	4 (5.4%)	1 (1.4%)		5 (62.5%)	0
Short neck	12 (40.0%)	0	16 (21.6%)	0		6 (75.0%)	2 (22.2%)
Scoliosis/ kyphosis	0	0	10 (13.5%)	1 (1.4%)		1 (12.5%)	0
<12 pairs of ribs	6 (20.0%)	0	1 (1.4%)	0		0	0
Short limbs	8 (26.7%)	2 (6.7%)	6 (8.1%)	3 (4.1%)	4 (40.0%)	0	3 (33.3%)
-Rhizomelic	2 (6.7%)	0	3 (4.1%)	0		0	2 (22.2%)
Short hands and feet	8 (26.7%)	3 (10.0%)	25 (33.8%)	1 (1.4%)		7 (87.5%)	3 (33.3%)
Polydactyly of fingers	1 (3.3%)	0	4 (5.4%)	0		0	1 (11.1%)
-Pre-axial	0	0	0	0		0	1 (11.1%)
-Post-axial	1 (3.3%)	0	2 (2.7%)	0		0	0
Clinodactyly	3 (10.0%)	0	7 (9.5%)	0		2 (25.0%)	0
Single palmar creases	4 (13.3%)	0	12 (16.2%)	2 (2.7%)		5 (62.5%)	4 (44.4%)
-Unilateral	4 (13.3%)		6 (8.1%)			1 (13.0%)	1 (11.1%)
-Bilateral	0		6 (8.1%)			3 (38.0%)	3 (33.3%)
Delayed bone age	1 (3.3%)	0	5 (6.8%)	1 (1.4%)		2 (25.0%)	0
Hypoplastic nails	7 (23.3%)	2 (6.7%)	7 (9.5%)	1 (1.4%)	1 (10.0%)	1 (13.0%)	1 (11.1%)
Polydactyly of toes	3 (10.0%)	0	4 (5.4%)	0		0	0
-Pre-axial	2 (6.7%)	0	4 (5.4%)	0		0	0
-Post-axial	1 (3.3%)	0	0	0		0	0

	Confirmed i(12p)					i(12p) not confirmed	Atypical karyotype
	Reported as neonates (n=30)		Reported when older (n=74)		Mathieu[52] paper		
	Present*	Absent*	Present*	Absent*	(n=10)	(n=8)	(n=9)
Hip dislocation	0	0	10 (13.5%)	0		2 (25.0%)	2 (22.2%)
Contractures	4 (13.3%)	0	11 (14.9%)	2 (2.7%)		0	1 (11.1%)
Features in thorax and abdomen							
Cardiac defects	7 (23.3%)	4 (13.3%)	19 (25.7%)	11 (14.9%)		0	3 (33.3%)
Accessory nipples	2 (6.7%)	2 (6.7%)	14 (18.9%)	12 (16.2%)		0	1 (11.1%)
Diaphragmatic hernia	18 (60.0%)	1 (3.3%)	1 (1.4%)	0		0	2 (22.2%)
Accessory spleens	5 (16.7%)	3 (10.0%)	0	4 (5.4%)		0	0
Exomphalos	4 (13.3%)	0	1 (1.4%)	25 (33.8%)		0	0
Umbilical hernia	0	0	18 (24.3%)	8 (10.8%)		2 (25.0%)	2 (22.2%)
Inguinal hernia	0	0	8 (10.8%)	1 (1.4%)		2 (25.0%)	1 (11.1%)
Imperforate anus	8 (26.7%)	3 (10.0%)	4 (5.4%)	3 (4.1%)		1 (12.5%)	1 (11.1%)
Hypospadias**	1 (6.7%)	3 (20.0%)	0	4 (9.5%)	1	0	0
Undescended testes**	7 (46.7%)	2 (13.3%)	13 (31.0%)	4 (9.5%)	1	2 (25.0%)	0

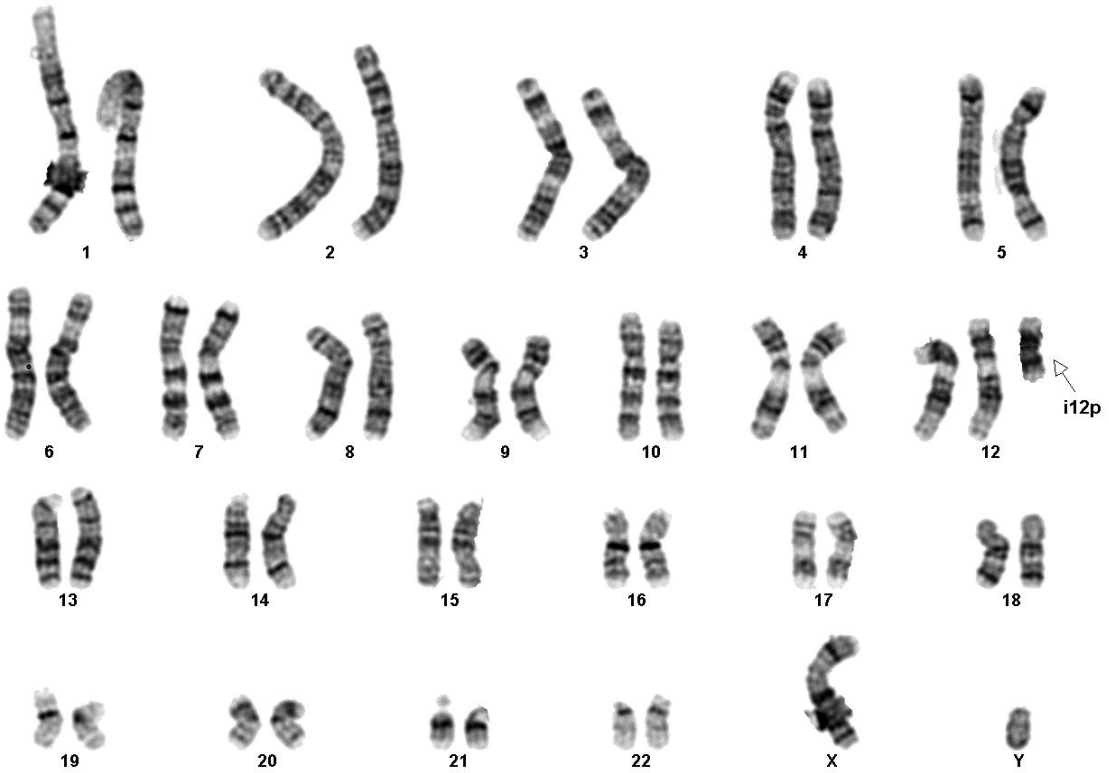
* The figures in the present and absent columns represent the cases where the literature report makes it clear that the feature was present or absent respectively. These figures rarely add up to 100% as few of the case reports contain all of the information sought for this literature summary.

**As these features are only seen in males, the percentages for these entries are calculated for the males in each group. In the Mathieu paper[52], the number of live-born males is not stated and the percentage cannot therefore be calculated.

1b: Genotype

Pallister-Killian syndrome is due to mosaic tetrasomy of 12p, normally caused by the presence of an isochromosome 12p, in addition to the two normal copies of chromosome 12, as shown in Figure 1 below. The isochromosome usually consists of two complete copies of 12p, separated by a chromosome 12 centromere. Chromosomal in situ suppression hybridisation has shown this to be smaller than the centromeres seen in normal copies of chromosome 12[36;90]. On rare occasions, mosaic hexasomy for the isochromosome 12p has been described[54;85;91].

Figure 1: Karyotype showing additional isochromosome 12p (arrowed)



The percentage of cells containing the isochromosome varies between individuals with Pallister-Killian syndrome. It is also dependant on the tissue examined. Reynolds et al[47] were able to detect the abnormal karyotype in only one of 10 patients when lymphocytes from blood samples were examined. However, 40- 100% of fibroblasts in the same patients showed the abnormal karyotype[47]. Similar findings were produced by another collaborative study[52], which included 10 affected children, with data relating to the pregnancy available in two of these, and another nine terminated pregnancies. This

study showed ranges of 10- 100% cells with abnormal karyotypes in skin fibroblasts, 0- 12.5% in blood samples and 0- 100% in amniocytes. Chen's review paper[92] shows ranges from 0- 100% for skin fibroblasts, 0- 30% for blood samples and 0- 88% for amniocytes. Cells containing the additional isochromosome have been reported to make up 0- 80% of fetal lymphocytes[36;93].

Some authors have proposed that in vivo selection causes the ratio of cells containing the isochromosome to those with a normal karyotype to decrease as the child gets older. This is difficult to study methodically due to the enormous variation between individuals and between tissues in the same individuals, and, as discussed later, differences in laboratory technique. However, Ward et al[53] found the isochromosome in 10% of lymphocytes from peripheral blood cultures taken at 3 days of age but did not detect the abnormality in any of 100 cells examined in the same child at 2 months of age, which would support this theory. Gamal et al[62] also showed a drop from 70% of buccal mucosa cells having the isochromosome 12p at one day of age to 40% at one year and then to 32% at 17 months. It is suggested that the small centromere found in the isochromosome makes it unstable and contributes to its loss but this has not been proven.

There are a small number of reports of children with mild or moderate mental retardation[18;20;23;24]. The isochromosome 12p was found in 37% of fibroblasts in one patient[18], 50% in a second[20], 55% in a third[23] and in 22.7% of buccal mucosa cells in the fourth[24]. These values are within the ranges seen in individuals with typical Pallister-Killian syndrome phenotype, so the ratio of skin or buccal cells with normal karyotypes to those with abnormal karyotypes does not predict the phenotype. This, and the age-related reduction in cells with the isochromosome, would suggest that the ratio of normal to abnormal cells does not influence the phenotype, although this seems counter-intuitive. It remains possible that either the ratio in certain tissues or the ratio at a certain stage of development alters the severity of the condition.

There are two case reports describing children who have the Pallister-Killian syndrome phenotype and who have mosaic tetrasomy of distal 12p but do not have the usual isochromosome 12p[82;94]. Each of them has an inverted duplicated marker chromosome, lacking the normal centromere. In one case, this consists of 12pter- p11.22 and, in the

other, of 12pter- p12.3 (see below for ideogram of 12p). These cases indicate that there may be a critical region for Pallister-Killian syndrome lying within the 12pter- p12.3 region. There is also a single case described with mosaicism for a ring chromosome containing two copies of 12p[95]. Unfortunately, the authors were unable to determine the telomeric breakpoint so the exact composition of the ring chromosome is unclear.

Figure 2: Ideogram of 12p

Other unusual forms of mosaic tetrasomy 12p include a mosaic inverted duplication of 12p, creating three copies of the 12p on one chromosome[15] and two cases where mosaicism for both trisomy 12p and an isochromosome 12p were detected[34;78]. Some other patients have had mosaic hexasomy for 12p, with two copies of the isochromosome 12p seen in some cells[54;85;91]. It is possible that other patients known to have mosaic tetrasomy 12p also have some hexasomic cells and this has simply not been recognised.

The karyotypes and clinical features seen in those who do not have the typical isochromosome 12p are summarised in Table 5 below and Table 27 on page 200 respectively.

Table 5: Atypical karyotypes reported as Pallister-Killian syndrome

G1[91]	Mosaic 48,XX +i(12p)+i(12p)/ 46,XX
G2[15]	Mosaic 46,XY, trp(12)(p11.2-p13)
G3[78]	Mosaic 47,XX +12p/ 47,XX +i(12p)/ 46,XX
G4[95]	Mosaic for supernumerary ring chromosome containing 2 copies of 12p
G5[82]	Mosaic tetrasomy for 12pter-12p11.2
G6[85]	Mosaic 47,XX +i(12p)/ 48,XX +i(12p)+i(12p)/ 46,XX
G7[34]	Mosaic 47,XX +12p/ 47,XX +i(12p)/ 46,XX
G8[54]	Mosaic 48,XX +i(12p)+i(12p)/ 46,XX
G9[94]	Mosaic tetrasomy for 12pter-12p12.3

Origin Of The Isochromosome

There has been little work to identify the origin of the isochromosome in this condition and no consistent answer can be found in the available reports. A number of different theories of isochromosome formation are available, as illustrated in Figure 3 (page 20). Most agree that the isochromosome most likely arises due to transverse, rather than longitudinal, division through the centromere. This would mean that the arms of the isochromosome should be identical, except for any regions where cross-overs had occurred. To produce a normal karyotype with an additional isochromosome, the centromeric mis-division would have to be accompanied by non-disjunction at some stage. Van Dyke et al[96] suggested that the centromeric mis-division occurs in meiosis I, causing subsequent non-disjunction. Rivera et al[97] agree that the centromeric mis-division comes first but suggest that it occurs in germinal premeiotic mitosis, with the non-disjunction occurring during meiosis I. Struthers et al[98] agree with this principle but in reverse order, suggesting that premeiotic non-disjunction is followed by centromeric mis-division in either meiosis I or meiosis II. Non-disjunction during meiosis II, followed by meiotic or postmeiotic mitotic centromeric mis-division[33] has also been suggested. The final theories involve mitotic error in the zygote, either in isolation[29], or because non-disjunction during meiosis has led to the formation of a trisomic gamete[99].

To try to elucidate the cause of the isochromosome formation, some authors have examined micro-satellite markers to determine the inheritance of the two copies of 12q

and the four copies of 12p. In three cases where normal bi-parental inheritance of 12q was proven, the presence of both maternal 12p alleles was shown[17;33]. Three further cases showed the presence of both maternal 12p alleles but the inheritance of the long arm of chromosome 12 was not elucidated[6;36;100]. These cases imply that the error occurred in the mother, with the ovum containing additional genetic material. The results could be due to a trisomic zygote, with the isochromosome formation being a secondary event, or due to a gamete containing the isochromosome 12p as well as a normal chromosome 12.

In another four cases, with proven bi-parental inheritance of 12q, the isochromosome contained the same alleles as one of the patient's normal chromosome 12s. In three instances the isochromosome material was of maternal origin[33;36;98] and in one case it was of paternal origin[36]. These cases give no indication whether the primary abnormality arose in the germ cell or the zygote.

De Ravel et al[99] were able to show the presence of a trisomy 12 cell line in fetal skin fibroblasts in a single case. The presence of an additional chromosome 12 in some cells and an isochromosome 12p in others supports the suggestion that the isochromosome formation is a post-zygotic event in response to the trisomy. In that case, maternal isodisomy for 12q was shown, with the analysis on 12p suggesting that both maternal 12p alleles were present. A single paternal allele was detected at each 12p marker examined and this was believed to represent the isochromosome.

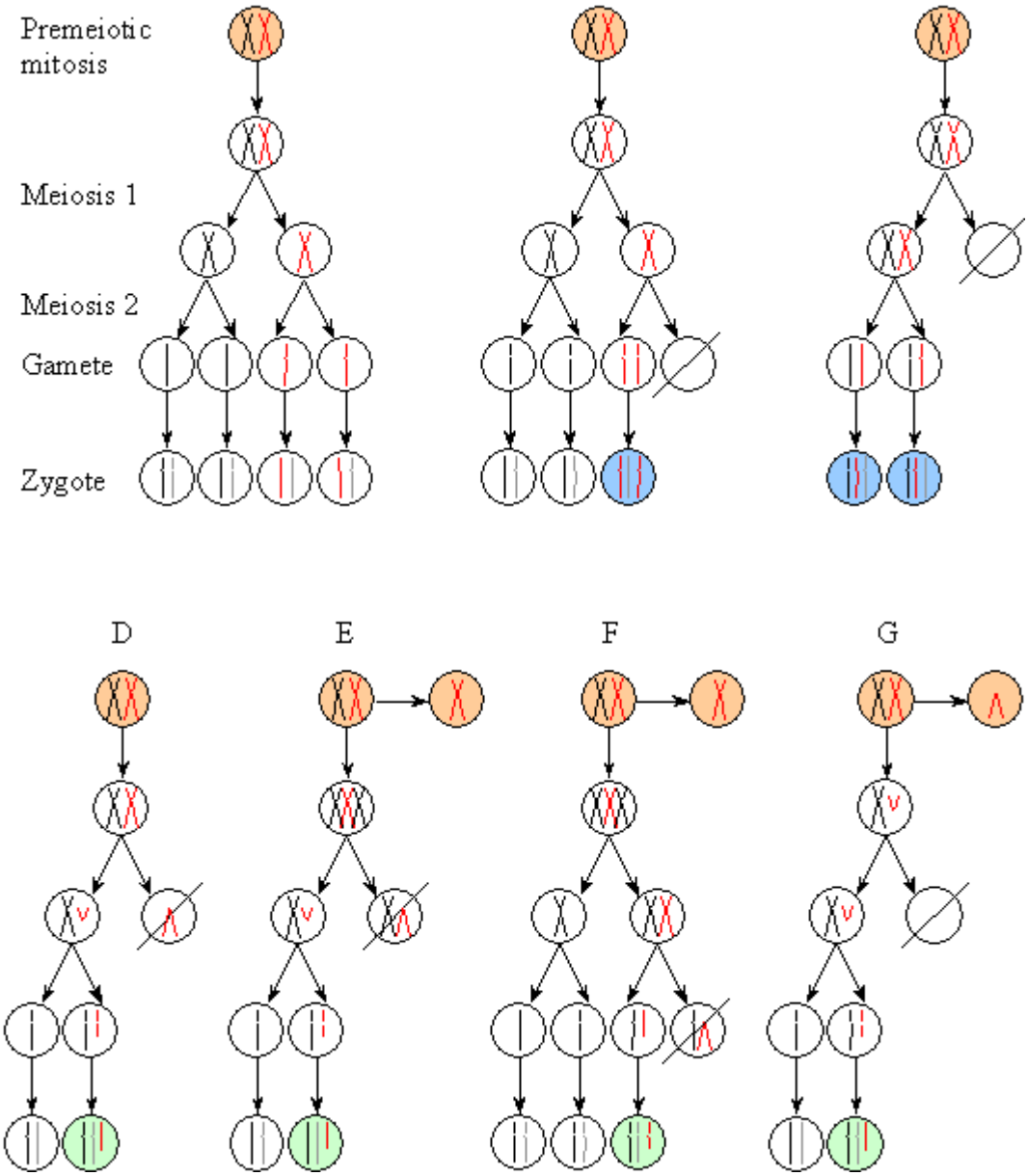
A further case reported by Chen et al[101] supports the theory of trisomy rescue. In that case, maternal heterodisomy was detected in the amnion, with no paternal copy of chromosome 12, but normal uniparental inheritance was shown in the chorionic villi. Fetal tissues showed the presence of one paternal and two maternal alleles on 12p with normal biparental inheritance of the 12q microsatellite marker.

Overall, although a number of families have been investigated, no clear answer on the origin of the isochromosome is yet available and further studies are required.

Relatively recent genetic studies have shown the presence of a number of imprinted genes in humans, where bi-parental inheritance is required for normal function.

Although no imprinted genes have yet been proven on chromosome 12, two genes on 12p and six on 12q are predicted to be imprinted[102]. It is therefore important to consider the possibility of a phenotypic difference between those with additional maternal and paternal 12p material. So far, none has been reported and the single reported case known to have uniparental isodisomy for 12q has a typical Pallister-Killian phenotype[99] but no research has formally considered this.

Figure 3: Postulated mechanisms of isochromosome formation



See over for key.

Key:

Blue-shaded zygotes (B- C) have three copies of chromosome 12 (trisomy 12), which predisposes to centromeric mis-division during mitosis and produces cells with an isochromosome 12p as well as two normal copies of chromosome 12.

Green-shaded zygotes (D- G) have two copies of chromosome 12 plus an additional isochromosome 12p. Subsequent loss of the isochromosome in some cells would explain the mosaic pattern seen in Pallister-Killian syndrome.

A- Normal process for reference

B- Non-dysjunction in meiosis 2 resulting in a disomic gamete and trisomic zygote.

C- Non-dysjunction in meiosis 1 resulting in a disomic gamete and trisomic zygote.

D- Centromeric mis-division in meiosis 1.

E- Non-dysjunction during premeiotic mitosis leading to a trisomic cell entering meiosis and predisposing to centromeric mis-division in meiosis 1.

F- Non-dysjunction during premeiotic mitosis leading to a trisomic cell entering meiosis and predisposing to centromeric mis-division in meiosis 2.

G- Centromeric mis-division during premeiotic mitosis leading to a cell with an isochromomse 12p and one normal copy of chromosome 12 entering meiosis, which predisposes to non-dysjunction in meiosis 1.

1c: Effect Of Increasing Parental Age

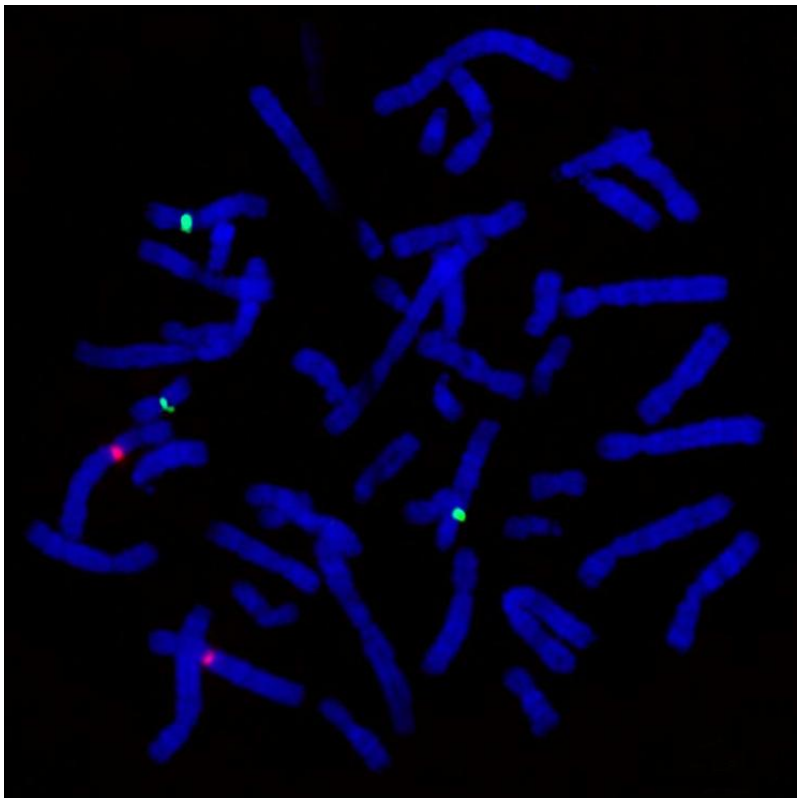
Wenger et al[103] saw a patient with Pallister-Killian syndrome, who was born to a 35 year old mother and a 52 year old father. This, along with the fact that increasing parental age is associated with chromosomal aneuploidy, led them to review the parental ages from published case reports available in 1988[103]. Their study supported the belief that increasing parental age leads to an increased risk of Pallister-Killian syndrome and the authors suggest that this is due to maternal, rather than paternal age. Although the small number (29) of informative reports quoted, the inclusion of their own patient and the assumption that United States maternal age norms could be applied worldwide cast doubt on the validity of the study, it has not been repeated. Further information on the origin of the isochromosome would be helpful in providing data on the biological plausibility to aid interpretation of studies like this.

1d: Methods Of Diagnosis

The isochromosome 12p is rarely seen in karyotypes performed on cultured lymphocytes so Pallister-Killian syndrome is rarely diagnosed on routine karyotyping. In part, this will be due to the low number of cells containing the isochromosome but selection against the cells containing the isochromosome 12p during the lymphocyte stimulation involved in karyotype preparation has also been demonstrated[104]. This process of selection has also been detected in other tissues, including bone marrow cells[53], amniocytes[57;105] and skin fibroblasts[28;56;60]. Tang et al[106] produced some evidence that this may be due to a higher rate of apoptosis in the cells containing the isochromosome 12p, although their data was not statistically significant and has not yet been replicated.

Pallister-Killian syndrome is usually diagnosed cytogenetically when the isochromosome is detected on a skin fibroblast karyotype, as shown in Figure 1 on page 15. The skin biopsy is obviously only carried out after clinical suspicion of the diagnosis, or at least of mosaicism. The isochromosome can, however, sometimes be detected by karyotyping samples from chorionic villi or amniotic fluid. In these cases, clinical suspicion of a chromosomal abnormality is all that is needed before testing is offered. A FISH karyotype is shown in Figure 4 overleaf.

Figure 4: FISH karyotype showing additional isochromosome 12p



Key:

Green signal: probe for chromosome 12 centromere

Red signal: probe for chromosome 6 centromere

This FISH karyotype shows 47 chromosomes, with the supernumerary chromosome containing a chromosome 12 centromere.

Pallister-Killian syndrome can also be diagnosed on buccal mucosa samples using FISH probes for the chromosome 12 centromere[19;107]. Since this does not require culturing of the sample, the potential in vitro selection against the cells with the abnormal karyotype is not a concern. Buccal mucosa sampling also removes the need for a skin biopsy and is therefore easier to perform in a clinical setting. Clearly, additional cytogenetic testing would be required to confirm that mosaic tetrasomy 12p, rather than mosaic trisomy 12 was present, if there was any clinical doubt.

Pallister-Killian syndrome has also been suspected on multiplex ligation-dependent probe amplification (MLPA) analysis of the sub-telomeric regions, when an increased dosage of the sub-telomeric region of chromosome 12p is detected. This testing cannot

differentiate mosaic tetrasomy 12p from trisomy 12p, which can be seen in unbalanced translocations. It has been superseded by array CGH in most centres.

With the recent development of array CGH technology, it may be possible to recognise Pallister-Killian syndrome on tissue[108;109] or blood samples[110;111]. This can be carried out on DNA extracted from whole blood, thereby removing concerns regarding selection against cells containing the isochromosome during lymphocyte stimulation. However, since individual cells cannot be investigated, this technology is unable to detect low-level mosaicism. Ballif et al[110] showed that their laboratory was able to detect 20% mosaicism but not 10% mosaicism for trisomy 21 using array CGH. Hoang et al[112] showed that their standard array CGH algorithm was able to detect 50% mosaicism for either trisomy 21 or trisomy 13 but unable to detect these trisomies at 25%. By adding in a second algorithm for analysis, they were able to detect these trisomies when only 10% mosaicism was present. This paper[112] also includes an intra-uterine death where the fetus was subsequently found to have Pallister-Killian syndrome following array CGH testing. It is estimated that 79% of cells contained the isochromosome. Theisen et al have recently reported a case where they were able to detect the presence of 18% mosaicism for partial tetrasomy of 12p using array CGH technology[113] and a report[114] comparing oligonucleotide arrays to BAC array mentions the detection of 10% mosaicism for i(12p). Clearly, it will be impossible to exclude a diagnosis of Pallister-Killian syndrome by these means unless the technology improves. Array CGH will also be unable to differentiate mosaic tetrasomy 12p from mosaic trisomy 12p and will be unable to confirm the presence of an isochromosome, rather than addition of 12p material to one of the chromosomes. Confirmation of the diagnosis by another means will therefore be required to guide the recurrence risk given to parents.

In some cases, Pallister-Killian syndrome has been detected on karyotyping from either a chorionic villus or an amniocentesis sample. The detection rate does appear to be better than from a blood karyotype but there are reports of normal chorionic villus sample karyotypes in affected fetuses[109;115]. In some cases, a subsequent karyotype from an amniocentesis sample has allowed a diagnosis to be made[115].

1e: Reasons For Study

The literature review confirmed that there was much still to understand about Pallister-Killian syndrome and further study was required.

No formal study into the Pallister-Killian phenotype had ever been reported and most of the information available was from single case reports. Almost all reported patients with Pallister-Killian syndrome have severe mental retardation, with the majority never learning to walk or talk. There are, however, a small number of reports of children with mild or moderate mental retardation[18;23;24]. It is unclear whether there is any way to predict which children will have a milder phenotype at an early, or prenatal, stage. Although many reported cases have details of the percentages of tetrasomic cells found in at least one cell type, each laboratory will have different procedures, so this data is not directly comparable. As all analyses performed during a study into the condition would use the same techniques and reagents, it should be clearer whether the percentage of tetrasomic cells has any impact on the phenotype. Analysing parental origin of the isochromosome would also determine whether the parent of origin has any effect on the phenotype, and, in conjunction with analysis of the parental ages at delivery should confirm whether there is a maternal age effect, as suggested by Wenger et al[103].

There are a number of questions families frequently ask when told that their child has a rare diagnosis, other than what it means for the child. The existing literature is unable to answer many of these, including the prevalence, recurrence risk and prognosis. There is no published indication of the prevalence of the condition, apart from the comment that it occurs in less than 1 per 10,000 births[1] or is rare. Equally, there is no evidence of the recurrence risk, either for another child with Pallister-Killian syndrome, or with other chromosomal disorders. The longevity of patients with Pallister-Killian syndrome is also unclear, and, as most reported cases are young children, it is difficult to predict the adult phenotype.

Although Pallister-Killian syndrome is rare, it is a condition that is often considered in the differential diagnosis and better information on the phenotype would aid the decision on if or when to offer a skin biopsy. Equally, better information on the ability of array CGH and buccal mucosa FISH analysis to detect the condition would be

helpful in this scenario. It is likely that many children with Pallister-Killian syndrome remain undiagnosed. Those without the reported classic features (such as high birth weight, high anterior hairline, thin hair in the frontotemporal region, diaphragmatic hernia, anal atresia, supernumerary nipples and hypopigmented/ hyperpigmented lesions that follow Blaschko's lines) are more likely to fall into this group. A formal study into the condition would be able to provide fuller data on the features seen in this condition.

2: STUDY AIMS

- To investigate the features and natural history of Pallister-Killian syndrome in a British cohort.
- To calculate the prevalence of the condition in live births in Great Britain.
- To confirm that the isochromosome consists of two whole copies of 12p.
- To determine the number of different 12p alleles and their parental origin.
- To investigate whether or not there is a maternal or paternal age effect in this condition.
- To determine whether array CGH analysis on blood samples is sufficient to make the diagnosis, without the need for a skin biopsy.
- To investigate whether there is any link between percentage mosaicism in blood, buccal mucosa or urine samples and the severity of the condition.

3: METHODS

3a: Literature Review

Literature searches were made on PubMed[116] using the terms 'Pallister-Killian', 'Pallister Killian' and 'tetrasomy 12p'. These searches were then combined using the 'or' function to ensure that each article identified by any one of these searches was displayed once. The abstracts, where available, were then reviewed.

A copy of each article describing at least one live born individual with Pallister-Killian syndrome was obtained, usually as an electronic copy. Where this was not possible, photocopies were obtained from a library. The references for each article studied were checked to ensure that any articles missed by the original literature search were identified and copies obtained.

The full text and photographs in each of these articles was reviewed and summary tables created showing the details recorded for each reported live-born case (see Table 21, Table 22 and Table 23). The cases in one article[52] that did not detail the cases individually were listed as a group in Table 24.

Articles describing terminated pregnancies or stillbirths where the fetus had Pallister-Killian syndrome were obtained if the abstract suggested that any unusual features had been found. Those that simply recorded that Pallister-Killian syndrome had been detected on a prenatal karyotype were not reviewed further.

The phenotypic data from the literature review was recorded in 1a: Phenotype, as well as the summary tables at the end of that section. These were updated throughout the study, as an automated weekly PubMed search identified any newly published articles.

3b: Study Preparation

After reviewing the literature on Pallister-Killian syndrome and deciding that a study into the condition was justified, I had to consider the practicalities of running the study and what information should be collected.

The first question was whether there were enough potential participants in the United Kingdom to allow worthwhile data to be produced. Since there had not been any population-based studies, the prevalence of the condition was unknown. I was able to confirm that Unique, the support group for patients and families with rare chromosomal disorders, had 20 patients in Great Britain registered on its membership database. Crude estimates of the potential number of participants were also obtained by extrapolation from the number of patients seen by the Wessex Clinical Genetics Service. A search through their clinical database and through the laboratory database revealed four patients diagnosed with Pallister-Killian syndrome, two of whom had subsequently died, in a population of approximately 2,900,000. Mid-2006 population figures for each country in the United Kingdom, the most recent available from the Office of National Statistics[117] at that stage and only available rounded to the nearest 100, are shown in Table 6. A simple calculation, shown below, produced the crude estimates shown in that table.

$$\frac{Area\ population}{Wessex\ population\ (2{,}900{,}000)} \quad x \quad Number\ of\ live\ Wessex\ patients \quad (2)$$

Table 6: Populations and potential participants

	Population	Estimate of potential participants
England	50,762,900	35
Wales	2,965,900	2
Scotland	5,116,900	4
Northern Ireland	1,741,600	1
Great Britain	58,845,700	41
United Kingdom	60,587,300	42

Although these are relatively small numbers and recruiting all potential participants was exceedingly unlikely, it still seemed worth performing the study. Only 11 patients with Pallister-Killian syndrome were required to make this the largest reported group of patients and previous articles include series of case reports rather than formal studies into the condition. Even though the estimate of potential participants was crude and the anticipated recruitment rate was approximately 50%, giving an estimated 20 participants in Great Britain, it seemed likely that a useful study could be performed.

The study required permission from national research ethics committees, the National Information Governance Board for Health and Social Care and Research & Development Units throughout the United Kingdom. In 2008, when this study was being planned, there was no unified United Kingdom ethical approval system in place. The amount of work required to duplicate the applications for each country therefore had to be considered along with the estimated number of potential participants for that country. Given the single estimated potential participant in Northern Ireland, that country was excluded and a study in Great Britain planned.

A protocol was then prepared, documenting the study aims, the information that would be collected from each family and the planned investigations. Careful consideration was given to how best to contact the potential participants, as it was important to contact as many as possible, without sending multiple invitations to any one individual or unintentionally contacting bereaved parents. Unique were happy to contact their patients directly and patients seen in the Wessex Clinical Genetics Service could also be contacted directly as I was part of the clinical team in that unit. Restricting the study to these groups would have reduced the possible number of patients recruited to the study and could have been viewed by a research ethics committee as discriminatory, since it would deny most families who had not joined Unique the opportunity to participate in the research. Since the services of the Medical Research Information Service were required to obtain details of those who had died, it seemed sensible to consider using their tracing service to contact patients. This would have allowed us to write to all carers of patients with Pallister-Killian syndrome in Great Britain via the Primary Care Trusts (England), Local Health Boards (Wales) or NHS Boards (Scotland) and then the General Practitioners. Advice was taken from the Medical Research Information Service, the Patient Information Advisory Group and the local research ethics

department, who all confirmed that this method of contacting the potential participants was acceptable.

Another major consideration was what to do with the research samples at the end of the study. Since Wessex Regional Genetics Laboratory did not hold a licence from the Human Tissue Authority to store research samples, the DNA samples should have been destroyed at the end of the study. This, however, did not seem ethical. It was possible that the study would produce results which indicated that further research was merited and it would have been inappropriate to take additional blood samples from these children for DNA extraction if the samples from this study could be used instead. It was therefore decided to offer parents the choice of destroying the DNA samples or storing them as clinical samples so that they would be available in the future. Since the research could potentially have identified non-paternity or confirmed that the child had inherited two copies of chromosome 12 from one of the parents, the decision was taken to label the samples using a unique research identifier initially. At the end of the study, the study identifiers on the samples that were to be retained in the NHS laboratory could be replaced by the participant's name and date of birth.

Consent forms were prepared for the study itself, for the laboratory investigations, for photographs of the patient and for parental samples. The photograph consent form included separate consents for the use of the photographs in medical conferences, in publications and for teaching purposes, to ensure no parent felt pressured into agreeing to any/ all of these. Staff at Unique were helpful in reviewing the protocol, patient information leaflet and consent forms and suggesting improvements. They also confirmed that the study did not put any unnecessary burdens on the participants or their families.

The next question was whether appropriate funding could be obtained in a timely fashion. I obtained confirmation that the Birth Defects Foundation, BDF Newlife, would be interested in providing funding for the study and that I could apply for a 'start-up' grant of up to £15,000 at any time. After submission of the formal application, a formal funding offer was received and accepted.

Research sponsorship was also required and a request was made to Southampton University Hospitals Trust via the R&D office. A provisional agreement was made promptly and this was finalised once all other approvals were in place.

An application for ethical approval was completed through the on-line 'Integrated Research Application System'[118]. Due to differences between the Mental Capacity Act 2005, which applies to England and Wales, and the Adults with Incapacity (Scotland) Act 2000, the application had to be submitted to two research ethics committees concurrently. The meeting date for the committee for England and Wales came first and they approved the study without any amendments. A few weeks later, the Scottish committee reviewed the study and asked that specific consent be sought to contact the families directly if further research into Pallister-Killian syndrome is planned. Although it would have been possible to use a different consent form for Scotland, obtaining consent to contact patients living in Scotland but not those in England or Wales would have been unhelpful due to the small numbers involved (see Table 6). It would also have been confusing, with the risk of using the wrong country's form for any given patient. The research ethics committee for England and Wales were therefore approached and accepted this amendment to the consent form.

As previously mentioned, the recruitment method had been carefully considered and discussed with all the relevant agencies. An application was submitted to the Patient Information Advisory Group because this involved obtaining patient-identifiable details (for example name and date of birth) without the specific consent of the patient or a carer. Despite the prior advice, the National Information Governance Board for Health and Social Care, which replaced the Patient Information Advisory Group after the application had been submitted, insisted that patient-identifiable information could only be supplied directly to the Medical Research Information Service. This made it much more complicated to obtain details of the causes of death and of the pre-natal diagnoses but it was still possible. Their proposed method of contacting the families of potential participants was, however, unfeasible. The National Information Governance Board for Health and Social Care wanted the cytogenetics laboratories to supply the patient details directly to the Medical Research Information Service, which would send out the study information sheets, along with a consent form. This would have prevented families from giving informed consent, as they could not discuss the study with a researcher

before signing the consent form and was therefore unacceptable. Although this was pointed out to the National Information Governance Board for Health and Social Care, they were unwilling to reconsider their decision so this method of recruitment had to be omitted.

R&D approval was sought shortly after the introduction of the 'National Institute for Health Research Coordinated System for gaining NHS Permission'[119]. Unfortunately the Trust R&D department had not up-dated its procedures, so both the old and the new documentation had to be completed and further new forms were introduced during the application process. Four months after the initial application had been submitted, R&D approval was granted and it was possible to start the main body of the study.

Finally, R&D approval had to be sought to access the laboratory data from the Scottish cytogenetics laboratories, as the National Institute for Health Research Coordinated System did not cover Scottish centres. Although no Scottish national approval existed at the time, the application was made through a coordinating centre, which performed some of the checks and forwarded the necessary information to the relevant Health Board R&D departments for formal approval.

3c: Recruitment And Epidemiology

Recruitment

Once all of the necessary permission letters had been received, recruitment commenced. The addresses of the patients with Pallister-Killian syndrome who had been seen in Wessex Clinical Genetics Service were checked on the NHS 'Personal Demographics Service' to ensure the information in their Genetics notes was current. The study invitation letter and patient information leaflet were then sent out along with a response form and a stamped, addressed envelope. Unique also wrote to all of their members who were eligible to participate in the study, enclosing the same information. To protect the confidentiality of the department's patients and of Unique's members, the two lists could not be compared, so it is possible that some families received the information from both sources.

The invitation letter asked families who were interested in participating to complete their contact details on the response form and return it to me. It also gave the option to indicate that they did not wish to participate and to prevent further correspondence. Each family who responded positively to the study invitation was contacted by telephone or email and given a chance to ask questions about the study. An appointment, usually in the patient's home, was then arranged at a mutually suitable time.

After a few months, Unique sent a reminder letter to their members who were eligible for the study but had not responded. To allow them to identify this group, a list of surnames of the Unique members was emailed to them after discussion on the telephone. No other patient identifiers were included and there was no mention of the study to ensure that the data would be meaningless to anyone who intercepted it. As mentioned above, it was not possible to check whether Unique would be writing to patients who had been seen in our department so no reminders were sent from the department.

Epidemiology

Towards the end of the study, each NHS cytogenetics laboratory in Great Britain was contacted by telephone. An explanation of the study was given and the laboratory staff

were asked whether that laboratory would be able to provide the required data. If so, an email was sent explaining the study in more detail and including copies of the National Information Governance Board for Health and Social Care consent and two data collection forms. Each laboratory was also provided with details of any patients who had been diagnosed in that laboratory and asked to exclude them from the data submitted. They were asked to complete a table with the dates of birth of live born patients, along with the age of the parents at delivery, if known, and to provide non-identifiable, clinical information on any cases that had been diagnosed either during pregnancy or after termination of pregnancy. Finally, they were requested to return a completed Excel spreadsheet to the Medical Research Information Service, containing the patient's name, date of birth, NHS number and address, along with the year that the address had been valid.

When these details reached the Medical Research Information Service, staff there checked whether the patient could be traced on their system. If so, they checked whether the patient had died or was still alive. For those who had died, the Medical Research Information Service supplied an edited copy of the death record, showing the date of birth, date of death and the cause(s) of death. The numbers of living patients and the numbers who could not be traced from the information given were also supplied, without any identifiable details.

3d: Phenotyping

At the start of the appointment with the family, written consent to participate in the study was obtained from a parent. Unless the family requested that the meeting run in a different order, a clinical and family history was taken next. A simple pedigree was drawn indicating the family birth order, spontaneous abortions and any chromosomal disorders in the patient's siblings. An Access database had been created to ensure that data was recorded consistently and this provided a structure to the remainder of the meeting. The clinical history started with the pregnancy details, including the use of any form of assisted reproductive technology, the presence of either polyhydramnios or oligohydramnios, whether fetal movements had been normal and whether any fetal or placental anomalies had been detected. To allow comparison with the data produced by the Office for National Statistics[120] and the General Register Office for Scotland[121], maternal and paternal ages at delivery were stratified into five year age bands and their marital status at that point recorded.

The gestation at delivery was recorded, along with the birth weight, length and head circumference if available. Where possible, weight, length and head circumference measurements at the ages of approximately two years, five years and 10 years were obtained from the personal child health record. The measurement taken closest to the specified age was recorded, as long as it was between 18 months and the third birthday (approximately two years), between the fourth and sixth birthdays (approximately five years), between the ninth and 11th birthdays (approximately 10 years). In cases where the exact dates and measurements figures were recorded, these were plotted using growth charts for the appropriate sex, based on the 1996 UK cross-sectional reference data, which was the standard at the start of this study. On occasions where a measurement had not been written down but had been plotted in the personal child health record, the plotted centile had to be accepted.

Parents were then asked whether the patient had been hypotonic or had feeding difficulties during infancy. They were specifically questioned about the presence or absence of congenital malformations that have been reported in Pallister-Killian syndrome, namely diaphragmatic hernia, cleft lip, cleft palate, imperforate anus and polydactyly. Enquiries were also made about other common findings, such as inguinal

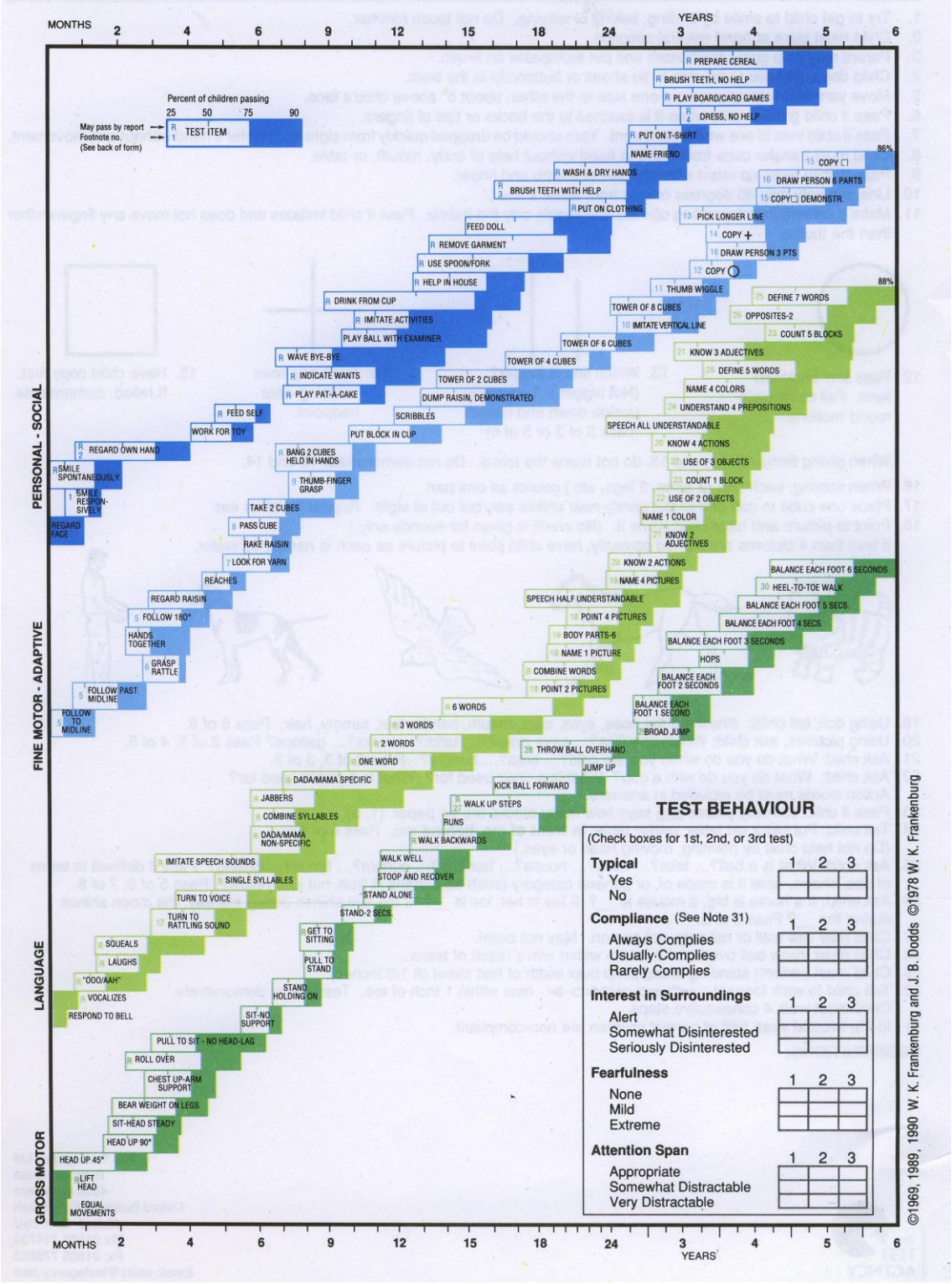
hernia, umbilical hernia, scoliosis, dislocation of the hip and cryptorchidism in males. If the patient had ever had an echocardiogram, renal imaging, cranial imaging, skeletal survey or chest X-ray this was noted. Since chest X-ray reports rarely mention the number of pairs of ribs present, the radiology department that had performed this investigation was requested to provide details of the number of ribs present or to supply a copy of the X-ray itself. For other investigations, results were obtained from the medical letters that the parents had or, if this was not possible, from the relevant hospital department at a later date. Seizures were discussed in some detail, with the age of onset, type of seizure, number of medications used concurrently and degree of seizure control recorded.

Enquiries were made about hearing and visual impairment and any specific abnormalities documented. Where possible, the age at which the first teeth erupted and some detail of the later dental eruptions was obtained. Although few parents know at what age their child's anterior fontanelle closed, this information was often retrieved from the parents' copies of the medical letters. Where that was not possible, parents were simply asked whether they remembered anyone commenting on delayed closure of the fontanelle. The presence of either recurrent or unusual infections was sought and parents asked whether the patient had a similar number of infections to his/ her siblings or contemporaries. In older children and adults, whatever details parents could recall regarding the onset of puberty were recorded to determine whether this occurred at the appropriate time. Details of sleep disturbance and the use of medication to aid sleep were noted. Open questions were posed about unusual or stereotypic behaviour and any other features that parents felt had not already been covered. This questioning resulted in information about anhydrosis in one of the first patients seen so this specific question was added to the database for all future patients.

To complete the history, parents were asked at what age the diagnosis had been made and which diagnostic tests had been carried out. If the parents had a copy of prenatal or skin biopsy results, the number of tetrasomic or hexasomic cells and the total number of cells visualised was recorded. Otherwise, the report was later requested from the issuing laboratory.

A simple developmental assessment was then performed, based on the Denver II developmental screening test, which is available in many paediatric textbooks and is shown in Figure 5. Information on key developmental milestones, such as smiling, sitting alone and walking was gained from parents' recollections or documentation in the parents' copies of medical letters, the personal child health record or other parental records. Current developmental age was calculated in the four separate fields (gross motor, fine motor/ adaptive, language and social/ personal) based on observations during the meeting and the information from the parents.

Figure 5: Denver II developmental screening test



After this, a dysmorphology examination was carried out on the patient. This was done in varying orders, depending on the patient's understanding and cooperation with the examination. Entering the data onto the Access database gave an opportunity to ensure that nothing had been missed. Any abnormalities of the head shape, cranial sutures or

forehead were recorded, along with the size of the anterior fontanelle in young children. Anterior and posterior hairlines, hair quantity, pigmentation and texture were examined and any areas of alopecia sought. The shape, volume and pigmentation of the eyebrows and eyelashes were reviewed, along with the slant and width of the palpebral fissures, inner canthal and inter-pupillary distances and presence of epicanthic folds, and any abnormalities of the iris, pupil or sclera were recorded. Nose formation was then considered, with comments on the length, prominence of the bridge, anteversion of the nares and any abnormalities of the tip or columella. Philtral length and prominence was assessed, along with mouth and lip shape and size. Abnormalities of the teeth, tongue and, where possible, the palate were also sought and the prominence of the jaw and ear formation, rotation and position considered.

Examination of the hands and feet covered the width, palmar and plantar creases, digit length, size of nails and the presence of either syndactyly or arrested polydactyly. The presence of a tri-phalangeal thumb or sandal gap was specifically sought and the length and proportions of the limbs considered. Finally, the presence of hypopigmented and/ or hyperpigmented skin lesions and of accessory nipples was sought.

At the end of the meeting, samples were taken for laboratory analysis, identified only by the patient's study number. A cytology brush was removed from its sterile plastic tube and rotated inside the patient's cheek for approximately 30 seconds to obtain buccal mucosa cells. The brush was then returned to its casing and 10ml of sterile 0.9% saline added to maintain the cells until the sample reached the laboratory. Parafilm was used to secure the cap of the brush into the tube to prevent leakage in transit. In the case of continent participants, parents were also asked to collect a urine sample from the patient into a sterile universal sample pot.

If the parents agreed to it and venous access was relatively easy, a blood sample was taken from the participant at the end of the meeting, using a 23 gauge winged infusion set ('butterfly'), and a 10ml syringe. The sample was stored in a K_3-EDTA blood bottle, labelled with the unique study identifier and secured within a protective plastic specimen holder. In cases where venous access was known to be very difficult or where parents would not permit blood sampling, verbal consent was sought to request a stored DNA sample from the relevant molecular genetics laboratory.

Assuming a sample was available from the patient or it was believed that a stored sample could be accessed, blood samples from both parents were requested. If only one parent was present and there was no reasonable chance of obtaining a sample from the other parent, then no parental samples were taken. Parental samples were taken directly into a K_3-EDTA blood bottle using a standard evacuated collection tube system once signed consent had been obtained. These samples were only identified as mother/ father and the patient's study number. In cases where one parent was not available at that point in time but was known, or believed, to be willing to participate, they were asked to approach their doctor's surgery to see if a practice nurse or General Practitioner could take the sample, or to use the phlebotomy services in a local hospital. A laboratory request card, study identification label, specimen holder and padded addressed envelope were provided to ensure there was no confusion over where the sample should be sent. The consent form was also left at the house with a separate envelope so that it was returned directly to the clinical department and did not arrive in the laboratory with the sample.

3e: Laboratory Analyses

DNA Extraction And Storage

DNA was extracted from the blood samples from the patients and their parents. This was a fully automated process using the chemagenic kit manufactured by Chemagen. The DNA samples were stored in the freezer in Wessex Regional Genetics Laboratory, in line with their usual procedures for clinical samples.

In cases where parents had been unhappy about a blood sample being taken from the patient, a stored sample was requested from the relevant clinical laboratory if available. These were then stored alongside the new samples in the laboratory.

At the end of the study, the samples that the families did not wish stored were disposed of as clinical waste. The samples that were to be retained had their Pallister-Killian study identifiers removed and were relabelled with the patient's or parent's name and date of birth. They were then returned to storage in the Wessex Regional Genetics laboratory freezers as clinical samples.

Array CGH Analysis

Once all of the Pallister-Killian patient DNA samples were available, they were removed from the freezer and thawed at room temperature. When completely thawed, the samples were thoroughly mixed using a vortex mixer and then centrifuged to ensure each sample was at the bottom of the sample tube.

Sample quality was then checked on the 'NanoDrop ND-1000 UV-VIS Spectrophotometer', which requires 1.3µl of each DNA sample. The machine draws the sample into a column and analyses the absorbance of ultraviolet light by the sample at different wavelengths. The associated software calculates the DNA concentration based on the absorbance at 260nm. It also calculates the ratio of the absorbance at 260nm to that at 280nm and the ratio of the absorbance at 260nm to that at 230nm. High quality DNA should have ratios of 1.8- 2.0 and above 2.0 respectively if the DNA is adequately free from contaminants. To ensure the DNA had not degraded, electrophoresis was performed on a 0.8% agarose gel and stained with the fluorescent dye, ethidium bromide. The gel was then photographed under ultraviolet light and the image checked

visually. The presence of a strong top band in each lane without smearing or additional small bands confirms that the DNA is reasonably intact.

Samples that passed the quality checks were spun in the centrifuge for one minute before 1.5µg was transferred to a microcentrifuge tube. A sex-matched control DNA sample was allocated to each patient DNA sample and treated in the same way. Distilled water was added to bring each sample up to a total volume of 20µl. The DNA was then digested using five units of each of the restriction enzymes, Alu I and Rsa I, along with two units of acetylated bovine serum albumin and additional buffer solution. After two hours' incubation in a water bath at 37ºC, the samples were heated to 65ºC for 20 minutes to inactivate the restriction enzymes.

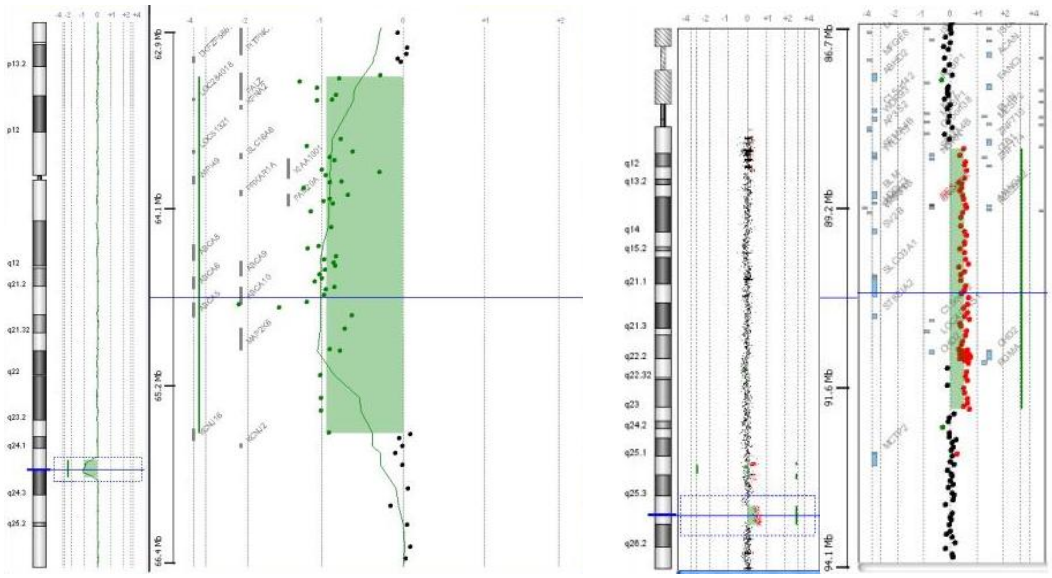
The samples were then labelled with fluorescent cyanine dyes, using a commercial Agilent DNA labelling kit. The patient samples were labelled with Cy5 and the control samples with Cy3. This was done by adding 5µl of the Random Primers mixture to the samples and incubating them at 95ºC for three minutes before adding 21µl of a solution containing buffer, the relevant cyanine dye and a DNA polymerase known as Klenow enzyme and incubating the mixture at 37ºC for two hours. After that period, the samples were heated to 65ºC for 10 minutes to inactivate the Klenow enzyme. They were then transferred to a filter that retains molecules larger than 30kDa, 430µl of buffer solution was added and the mixture was centrifuged. The procedure was repeated a second time before the purified sample was collected. The yield and specific activity of the labelled genomic DNA were then measured on the NanoDrop ND-1000 UV-VIS Spectrophotometer.

Each patient DNA sample was then mixed with the corresponding control DNA sample. 5µg of Cot-1 DNA, along with a commercial blocking agent and hybridisation buffer, was added to bind the highly repetitive DNA sequences and prevent non-specific hybridisation. This required incubation at 95ºC for three minutes and then at 37ºC for 30 minutes, after which the mixture was centrifuged for one minute. A hybridisation gasket slide was fitted into the base of its chamber and 100µl of the sample mixture pipetted into one of its four wells. Once each well contained a sample, a microarray slide, to which the DNA should hybridise, was placed on top of the gasket slide and the chamber cover secured. The samples were then placed in a hybridisation oven set at

65ºC with a speed of 20 rotations per minute for 24 hours. The slides were then removed from the chamber and the gasket slide separated from the microarray slide. The microarray slides were placed into a bath of wash buffer solution for five minutes at room temperature and then transferred to a second wash buffer solution at 37ºC for a further minute. The slides were then removed from the solution and allowed to dry before being inserted into slide holders and placed in the Agilent scanner. Laser beams were passed across the slides, causing the cyanine dyes to fluoresce and the relative intensities of the Cy3 dye, which appears green, and the Cy5 dye, which appears red, at each probe read by the software. The results were displayed on a $\log_2$ scale both numerically and pictorially by chromosome region, as shown in Figure 6 below. Due to the $\log_2$ scale, a hemizygous deletion should be displayed at -1 and a hemizygous duplication displayed at 0.58.

Where the array CGH detected copy number variants that are not recognised polymorphisms, a DECIPHER[122] report was included with the array CGH result, as per standard laboratory practice. This lists all known genes in the deleted or duplicated region. Where required, the functions of these genes were checked on the Online Mendelian Inheritance in Man website (OMIM)[123] and the relevant articles obtained for review. If no entry was found on OMIM under any of the aliases, a further search was made on the PubMed website[116]. If no data could be found on either, it was accepted that the gene was not known to cause disease. The deletions/ duplications were also checked against the DECIPHER[122] database, the database of genomic variants[124] and on the PubMed website[116] to see if they were known to be associated with a clinical phenotype or likely polymorphisms.

Figure 6: Example of array deletion and duplication



Key:

Black dots: probes where the intensities of Cy3 and Cy5 fluorescence are similar

Green dots: probes where the intensity of Cy3 fluorescence is higher than of Cy5 (suggesting that the patient has a deletion in that region)

Red dots: probes where the intensity of Cy5 fluorescence is higher than of Cy3 (suggesting that the patient has a duplication in that region)

In each case the ideogram on the left shows the full length of the chromosome, with the blue line representing the centre of the region enlarged on the right. The dotted blue box in the middle figure denotes the full region enlarged on the right.

Note that the green dots in the first figure approximate –1 on the $\log_2$ scale, indicating a hemizygous deletion, while the red dots in the second figure approximate 0.58 on the $\log_2$ scale, indicating a hemizygous duplication.

<u>FISH Analysis</u>

Each buccal mucosa sample arrived at the laboratory in a transport tube filled with 0.9% saline, which was first transferred into a 10ml conical base test tube. The brush used to obtain the sample was then shaken in fluid to dislodge any cells attached to it before it was discarded. Approximately 2.5ml of a fix solution (three parts of methanol to one part of acetic acid) was used to rinse out the transport tube. This solution was added to the test tube and the process repeated.

Once the resulting mixture had been spun on a centrifuge at 13000 rotations per minute for five minutes, the saline and fix solution mixture was carefully removed, leaving the buccal cells at the bottom of the test tube. A further 5ml of fix solution was added and the process repeated twice, after which a small amount of the fix solution was added to create a thick cell suspension.

The initial treatment of the urine samples was slightly different. They arrived at the laboratory in a sample bottle and each sample was split between two 10ml test tubes. These were spun on a centrifuge at 1500 rotations per minute for five minutes. The supernatant was then poured out of each test tube, 2ml of the fix solution added to each and the two samples combined so that there was only one sample per patient.

After this, the procedure for the buccal and urine samples was identical. A glass pasture pipette was used to drop a small amount of the cell suspension onto a clean dry slide, which was then allowed to dry. Further cells or fix solution were added as required. One additional drop of fix solution was then added to the slide and the slide allowed to dry. Once this had happened, the slide was exposed to ultra violet light for 15 seconds and then treated with 70µl of an RNA/pepsin solution under a coverslip for 2½ minutes at room temperature. (The RNA/ pepsin solution was prepared by diluting 50µl of a stock solution of each in 450µl of a 2x saline-sodium citrate buffer.) The slide was then washed in a 2x saline-sodium citrate buffer for two minutes at room temperature. It was then dehydrated through an alcohol series of 70%, 90%, 100% ethanol, each for two minutes and allowed to dry in the air.

A probe mix was created containing the pα128 probe, which binds to the chromosome 12 centromere and produces a red signal, and the p308 probe, which binds to the chromosome 6 centromere and produces a green signal. The p308 probe acted as a control. After 3µl of this probe mix had been added to the slide, it was covered with a coverslip and sealed with rubber cement. The slide was then heated to 73^{o}C on a hot plate for five minutes and incubated at 37 ^{o}C for four hours.

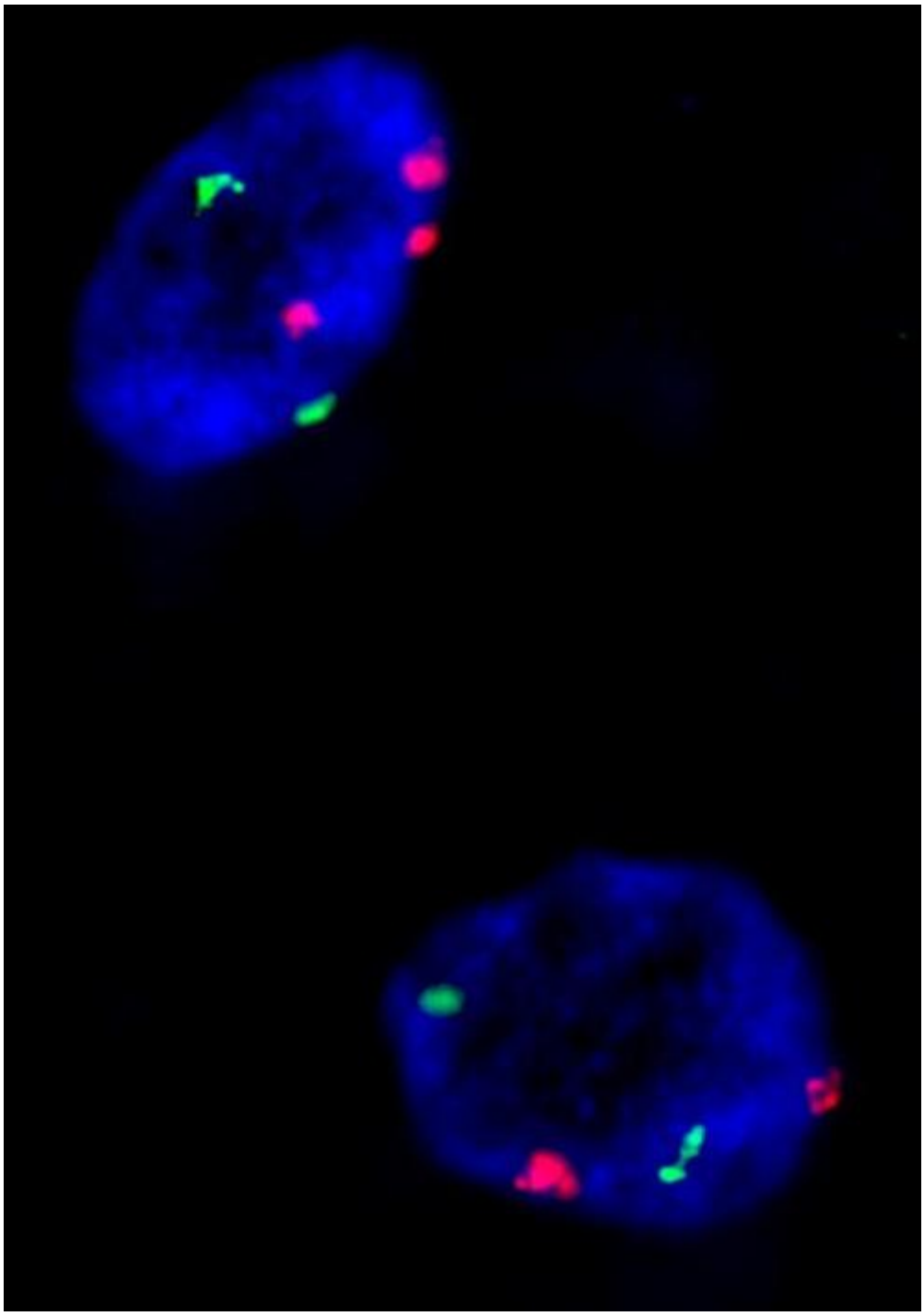
The coverslip was removed and the slide transferred to a 2x saline-sodium citrate buffer for two minutes. It was then transferred into a slide holder containing a 50% formamide and a 1x saline-sodium citrate buffer stringent wash, at 42 ^{o}C for five minutes, shaken,

and given another five minutes. After this, the slide was transferred to a wash buffer solution of 4x saline-sodium citrate buffer and 0.2% Tween 20 (polyethylene glycol sorbitan monolaurate) for two minutes. This was repeated with a fresh 4x saline-sodium citrate buffer and 0.2% Tween 20 solution for another period of two minutes.

After this, 60µl of a detection reagent made of 1:100 tetramethylrhodamine-5-isothiocyanate, 1:500 fluorescein isothiocyanate and blocking solution was added to a coverslip. The slide with the buccal cells was then placed onto the coverslip, so that it completely covered them and that no air bubbles were present. This was then incubated at 37 ^{o}C for 15 minutes, before being placed into 4x saline-sodium citrate buffer for two minutes.

4',6-diamidino-2-phenylindole was added and the slide sealed with clear nail varnish. Each slide was then reviewed under a florescent microscope, as shown in Figure 7 and the number of chromosome 12 centromere signals seen per cell noted. Where possible, 100 cells were analysed per patient.

Figure 7: Example of buccal FISH analysis



Key:

Red signal: probe for chromosome 12 centromere

Green signal: probe for chromosome 6 centromere

The upper cell shows three discrete red signals, proving that there are three copies of the chromosome 12 centromere in that cell. The lower cell shows the normal two signals for each centromere.

Microsatellite Analysis

Where feasible, samples were obtained from each patient and both parents and DNA extracted, as documented in previous section. A range of microsatellite markers covering 12p and, to a lesser degree, 12q were identified, as shown in Table 7.

Table 7: Position of microsatellite markers

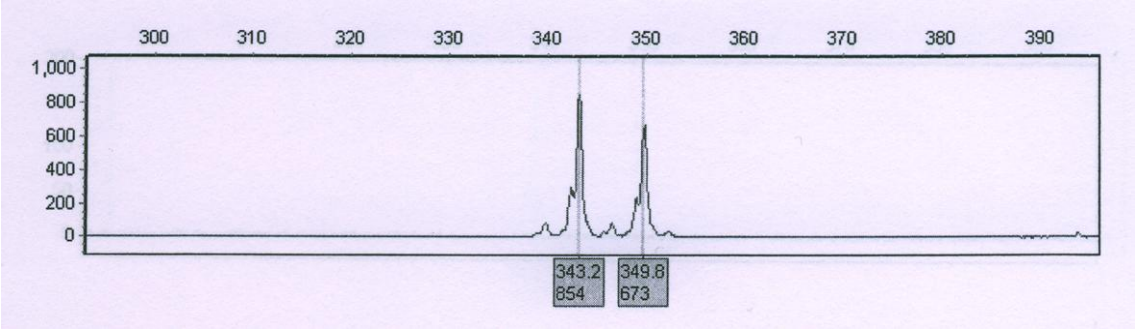
Microsatellite markers	Position on chromosome 12
D12S94	12p13.33
D12S1608	12p13.33
D12S1694	12p13.33
D12S1725	12p13.32
D12S1692	12p11.1
D12S1635	12q13.13
D12S1598	12q21.33
D12S819	12q21.33

Polymerase chain reaction (PCR) was used to amplify each microsatellite marker separately. First, 5µl (approximately 50ng) of the patient's/ parent's DNA was mixed with 0.2µl (2 micromoles) of deoxynucleoside triphosphates, 0.2µl (2 micromoles) of magnesium chloride, 0.05µl of QIAGEN's 'HotStarTaq' polymerase (0.25 units of Taq), 1.0µl of QIAGEN's 10 times PCR buffer and 25ng each of forward and reverse primers. One of the two primers in each set was labelled with either a FAM fluorescent tag or a HEX fluorescent tag. Deionised water (2.8µl) was then added to give a reaction volume of 10 µl.

The PCR mixtures were incubated in a thermal cycler for 15 minutes at 94ºC to activate the Taq polymerase, followed by 30 amplification cycles of 94ºC for 30 seconds to allow denaturing of the DNA, 55ºC for 30 seconds to allow the primers to anneal to the single-stranded DNA template and 72ºC for 30 seconds to allow synthesis of a new complementary strand of DNA. The thermal cycler continued for five minutes at 72ºC to allow completion of any unfinished DNA strands and 10 minutes at 60ºC to allow standardisation of the product lengths. Completed reactions were held at 15ºC until removed from the thermal cycler.

The mixture was then transferred to the Applied Biosystems 3130 Genetic Analyser for capillary electrophoresis to provide data on the alleles present and the peak sizes. This data was then exported to GeneMarker 1.85 software for analysis. Each patient's sample was analysed to see how many different alleles were present at each locus. As smaller alleles amplify preferentially (see below), a direct comparison of the peak heights could not be performed. Instead, the relative peak heights were compared visually to the normal peak profile of the patient's parents. Where parental samples were not available, other parental samples with similar allele sizes were used.

Figure 8: Example of microsatellite analysis, showing preferential amplification of smaller allele



The grey vertical lines indicate peaks that have been identified by the GeneMarker 1.85 software. Note that the larger (349.8) allele has produced a lower peak than the 343.2 allele in a normal control sample.

3f: Data Analysis

Birth and death data was obtained from the websites of the Office of National Statistics[120;125], which covers England and Wales, and from the General Register Office for Scotland[121]. The birth data provided a breakdown of the numbers of live-born babies by year of birth, marital status of parents, maternal age at delivery and paternal age at delivery. The ages of both parents are stratified into identical five-year age bands on both websites, although the Scottish one does have an additional column for 'unspecified'. Death data can be obtained showing the numbers of deaths in the neonatal period, up to one year of age, one to four years of age and then in five yearly bands, until 80 to 84 years, with the final band being 'at least 85 years of age'.

The population prevalence was calculated by adding the number of patients seen as part of the study to the number of living patients who had not participated in the study, as identified by the cytogenetics laboratories and the Medical Research Information Service. This figure was then divided by the population of Great Britain, as identified from the Office of National Statistics website[117] and multiplied by one million to give the population prevalence as a per million figure, as shown below.

Figure 9: Calculation of population prevalence

$$\frac{\textit{Number of living patients identified}\ (36)}{\textit{Population of Great Briatain}\ (58{,}845{,}700)} \quad \text{X} \quad 1{,}000{,}000$$

The British birth incidence for Pallister-Killian syndrome in live births was calculated over the most recent five-year period (2005 to 2009), as the data accessible by the cytogenetics laboratories is likely to be most complete for this period. The data from the Office of National Statistics[120] and the General Register Office for Scotland[121] was used to identify the total number of live births in Great Britain over that period, which provides the denominator. The numerator was the number of live births of babies with Pallister-Killian syndrome during this period, as identified from the data supplied by the cytogenetics laboratories added to those who participated in the study. The calculated figure was then multiplied by one million to give the birth incidence per million, as shown below.

Figure 10: Calculation of birth incidence

$$\frac{Number\ of\ Pallister-Killian\ patients\ born\ alive\ during\ period\ (19)}{Number\ of\ live\ births\ during\ period\ (estimated\ 3{,}707{,}352)} \quad X \quad 1{,}000{,}000$$

Data from the Office of National Statistics[120] was also used to identify the number of males and females living in Great Britain. The sex ratio found in the study was compared to the population data using a Chi-squared test with one degree of freedom and a Yates correction. Statistical significance was accepted if the probability value was less than 0.01. The first step in the calculation is to tabulate the data into a 2 x 2 table, as shown in Table 8. The total (N) can be calculated by adding either S + P or M + F, as both should give the same answer. The same procedure was used for other analyses where statistical significance was analysed using a Chi-squared test. Statistical help came from a medical statistics book[126].

Table 8: 2 x 2 table for chi^2 analysis of sex ratios

Males in study (MS)	Females in study (FS)	Total in study (S)
Males not in study (MP)	Females not in study (FP)	Total not in study (P)
Total males (M)	Total females (F)	Total (N)

Figure 11: Chi^2 analysis

$$Chi^2 = \frac{N\ (|MS \times FP - FS \times MP| - 0.5 - 0.5N)^2}{S \times P \times M \times F}$$

Data from the Office of National Statistics[120] for the year 2005 included additional statistical tables giving a breakdown of live births by gestational age. This was not available for any other year so these figures were used when comparing the number of premature deliveries in the study group to those in the general population.

For analysis of the death rates, a Kaplan-Meier survival curve, the usual way to display death data, was not suitable for this study because of the small numbers involved, especially in the older age groups, but a relative risk analysis was possible. To do this, the most recent Office of National Statistics[120] data, which was for deaths in 2009, was used as the population norm, along with the same year's population estimates.

Although the Office of National Statistics data on deaths is stratified into those aged less than one year, aged one to four years and then in five-year age bands, these were amalgamated into decades due to the small numbers. This reduced the risk of producing statistically significant results purely because of the small sample number. The relative risk of death in the Pallister-Killian syndrome group was compared to the population group up to the age group of the oldest known Pallister-Killian syndrome patient. An additional analysis was carried out on deaths under the age of 20 years, as an approximation of the risk of death in childhood.

The relative risk calculation involves creating a 2 x 2 table, in the same way as shown above (Table 8) for the chi-squared analysis. The table below (Table 9) was used for each separate age range.

Table 9: 2 x 2 table for relative risk analysis

PKS deaths (SD)	Population deaths, excluding PKS (PD)	Total deaths (D)
Living PKS patients (SL)	Living population, excluding PKS (PL)	Total living (L)
Total PKS patients (S)	Total population, excluding PKS (P)	Total population (N)

The relative risk was then calculated as shown in Figure 12 and a 95% confidence interval calculated as shown in Figure 13 and Figure 14.

Figure 12: Relative risk calculation

$$RR = \frac{SD/S}{PD/P}$$

The standard error of the logarithm of this relative risk then has to be calculated, as shown in Figure 13, as it is required when calculating the 95% confidence interval.

Figure 13: Calculation of standard error of the logarithm of the relative risk

$$SE(\log_e RR) = \sqrt{\frac{1}{SD} - \frac{1}{SD+SL} + \frac{1}{PD} - \frac{1}{PD+PL}}$$

The 95% confidence interval is then calculated as shown in Figure 14 below.

Figure 14: Calculation of the 95% confidence interval for the relative risk

95% CI= antilog$_e$ (log$_e$ RR – 1.96SE (log$_e$ RR)) to antilog$_e$ (log$_e$ RR + 1.96SE (log$_e$ RR))

Growth parameters were compared to growth charts for the appropriate sex, based on the 1996 UK cross-sectional reference data. Where possible, exact measurements were recorded during the meeting with the family but, where this was not possible, the nearest centile was recorded instead. For example, measurements above the 50th centile but closer to it than the 75th centile (those up to the 62.5th centile) were classed as 50th centile measurements. Retrospectively, it would have been easier to analyse the data if the centile line above the patient's measurement had been recorded for those between centile lines. To correct for the way the data had been recorded, the mid-point between each pair of centiles had to be calculated, as shown in Table 1, and used when graphing the expected centiles.

Table 10: Mid-points on centile charts

Centile	0.4th	2nd	9th	25th	50th	75th	91st	98th	99.6th
Mid-point	1.2	5.5	17.0	37.5	62.5	83.0	94.5	98.8	

Developmental age was calculated separately for gross motor, fine motor/ adaptive, language and social/ personal skills. The age at which 50% of children attain the highest-level skill that the patient had achieved in each category, as per the Denver II developmental screening test charts, was recorded as the developmental age for that category. An overall developmental age was not calculated as many of the patients had marked differences between their abilities for the different developmental categories.

In some sections, the group has been split into three sub-groups to segregate those with a milder phenotype, a moderate phenotype and a severe phenotype. Those who walked before their third birthdays and had good verbal communication skills were included in the milder phenotype group, along with the younger children who were developmentally normal at the time of assessment. The severe phenotype group constitutes those who by the age of three years had no speech sounds (including babbling or imitating speech) and could not sit unsupported. One younger child who, at 22 months, lacked head control when supported in a sitting position and had no speech sounds was also included in this group. The others were classed as having a 'moderate' Pallister-Killian syndrome phenotype.

Normal ranges for physical characteristics other than weight, length/ height and head circumference were obtained from the 'Handbook of Physical Measurements'[127]. Any measurements outside the given ranges were classified as abnormal. Although they were not published at the time that this study was planned, dysmorphic features have, where possible, been defined in the terms given in a series of articles that attempt to standardise dysmorphology terminology[128-133].

The 1998 Office of National Statistics data was used as the norm for parental age at delivery, as this as the most recent year where full data was available for the fathers. A spreadsheet was created showing the maternal age in the above mentioned 5-year age bands at the time of delivery in one column. For the study patients, the second column then indicated that the baby was affected. An additional worksheet was created with the population live-birth data and the second column here indicated that the baby was not affected. These worksheets were then merged within SPSS 17 and the age bands and affected status transformed to the required numerical coding.

First, a crosstabs analysis was performed to ensure the data had all been entered and transformed correctly. Once this had been confirmed, a binary regression analysis was performed, using the affected status as the dependent variable and maternal age as the independent variable. Since the literature suggestion was that the risk of Pallister-Killian syndrome increases with maternal age, the youngest group of mothers was used as the reference category.

This analysis was performed for the maternal age at delivery of those who participated in the study and then again, including the maternal age data collected from the laboratories as well. Paternal age analyses were performed in the same manner, again using the data for those who had participated in the study and then using all available data. Due to the small numbers of affected individuals, each analysis was then repeated after further grouping of the parental age bands.

4: RESULTS

4a: Recruitment

Twenty-four families were recruited to the study. In 21 of these, there was a living patient with mosaic tetrasomy 12p. The other three families could only be included in parts of the study and are detailed below.

In one case, the patient had recently died but his parents provided the history and photographs of the patient for review. The buccal mucosa samples, blood samples and much of the clinical examination could not be performed but he has been included in the other sections of the study.

The second family had undergone termination of pregnancy after receiving a pre-natal diagnosis of Pallister-Killian syndrome. When the diagnosis was explained to them, the study was also mentioned and they requested to participate in it. Blood and buccal mucosa samples could not be obtained but other fetal tissues were examined. They have therefore been included in the pregnancy details and in the microsatellite analysis.

The final case had an unbalanced translocation leading to trisomy 12p in all cells, rather than the mosaic tetrasomy 12p seen in Pallister-Killian syndrome and was referred to the study by a Clinical Genetics department. It is interesting to compare his phenotype to that of the children with Pallister-Killian syndrome but his details have to be considered separately so he has been excluded from the data analysis.

As previously mentioned, Unique contacted the families of 20 living patients with mosaic tetrasomy 12p and 15 of them (75%) participated in the study. Two families with a living child with Pallister-Killian syndrome were contacted directly from the Wessex Clinical Genetics Service, with one (50%) accepting the study invitation, and another three heard about the study from a different Clinical Genetics department. Some families were told about the study by their Clinical Genetics department after being recruited through Unique so it is not possible to say how many of the participants could potentially have been recruited this way. Some unexpected recruitment also came through the families themselves, as a number of them had made contact with other

families, either with the help of their local Genetics service or through international on-line support groups. Two (8.3%) of the families with a living child with Pallister-Killian syndrome were recruited to the study after hearing about it purely through other families.

Eight of the 22 live-born patients with Pallister-Killian syndrome (36.4%) were female and 14 (63.6%) were male. The chi-squared value for the difference between these values and the total population values is 1.34, which equates to a probability value of 0.25. If the population values for those aged 31 years or less are used to match the study group's age range, the chi-squared is 0.95 and probability value is 0.33. The difference in the sex ratio seen in the study group is not therefore statistically significant.

The youngest was seen at four months old and the oldest at 31 years of age. Half of them were under five years of age, one was an adolescent (12- 17 years old) and three were adults (at least 18 years old). The age and sex breakdown of the participants is shown in Figure 15 below. This is are broadly in keeping with the age range beyond the neonatal period seen in the literature, as shown in Figure 16 below.

Figure 15: Schematic showing age and sex of participants

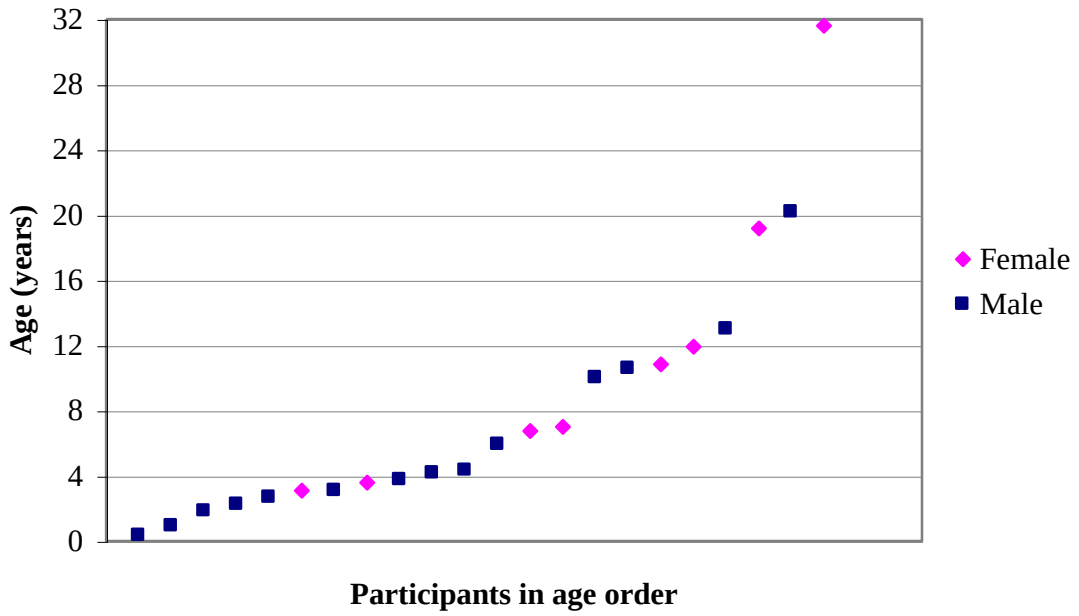
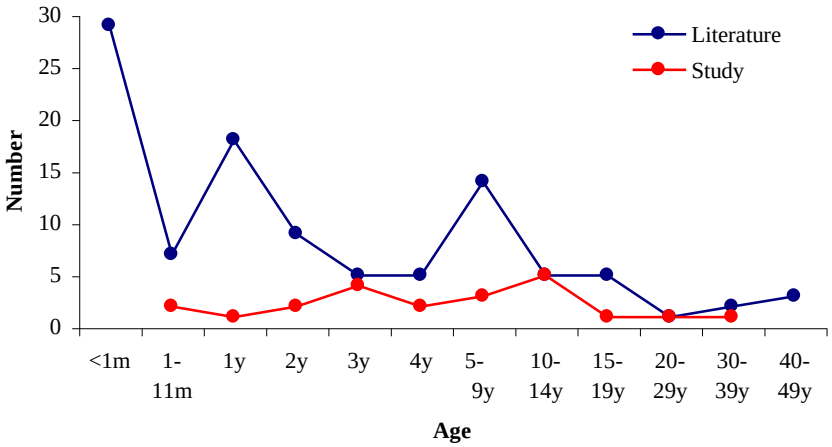


Figure 16: Age range in study compared to population



Most of the British cytogenetics laboratories (18 out of 22; 81.8%) were able to provide details of the patients diagnosed with Pallister-Killian syndrome in that laboratory. This data identified 25 other live-born individuals with Pallister-Killian syndrome, 15 of whom were still alive and in Great Britain. Another six were identified as being deceased. One had moved abroad and the other three could not be identified by the Medical Research Information Service from the information provided, so it is not possible to confirm whether these individuals are still alive.

Overall 58.3% of the identified patients with Pallister-Killian syndrome living in Great Britain participated in the study (21 out of a possible 36). This figure is similar to the estimated 41 potential participants calculated during the planning phase of the project, as shown in Table 6: Populations and potential participants.

4b: Epidemiology

As well as the 21 living patients with Pallister-Killian syndrome recruited to the study, another 15 in Great Britain were identified by the Medical Research Information Service from the information supplied by the cytogenetics laboratories. This equates to a population prevalence of 0.6 cases per million. The calculated British birth incidence for Pallister-Killian syndrome in live births over the five year period from 2005 to 2009 is 5.1 per million.

The study identified eight deceased patients in total. These include the boy whose parents chose to participate in the study after his death and another participant who died a few months after the study meeting. In one case, the data the cytogenetics laboratory supplied to the Medical Research Information Service stated that the patient had died but the Medical Research Information Service were unable to trace the patient and therefore could not provide the age at death or a copy of the death certificate. Given the date of birth provided by that laboratory and the date of the enquiry, the patient must have been aged less than 20 years at the time of death.

For the seven where the data could be obtained, the patients had died at the ages of one hour, four years (two patients), 10 years, 20 years and 38 years (two patients). The baby who died aged one hour had been delivered at 21 weeks gestation and the cause of death was this extreme prematurity. One death was classified as an unexpected death in epilepsy (20 years old) and another followed aspiration of the gastric contents (38 years old). The cause of death in the other four cases was a respiratory infection.

The relative risks of death in Pallister-Killian syndrome in comparison to the general population are shown in Table 11 below. In each case the 95% confidence interval starts above zero, showing that the results are statistically significant. Due to the small numbers involved, especially in the older age groups, this table assumes that each living patient with Pallister-Killian syndrome will remain alive until reaching the specified age group. The relative risks may therefore be underestimated in all cases.

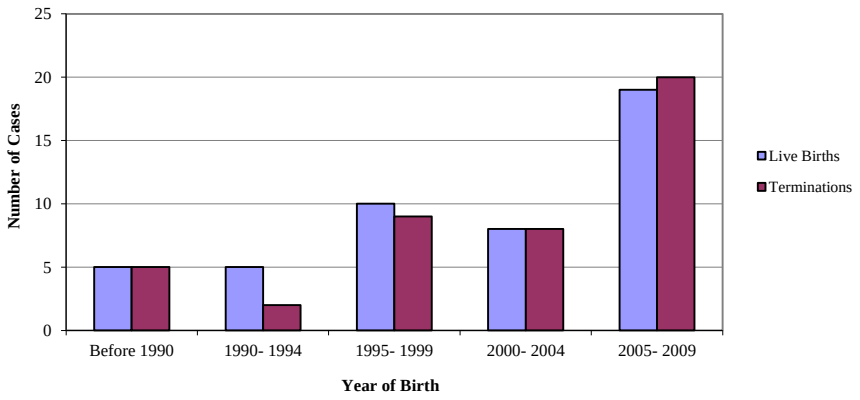
Table 11: Relative risks for death in Pallister-Killian syndrome

Age (years)	Population	Population deaths	PKS patients	PKS deaths	Relative risk (95% confidence interval)
<10	6,391,591	4,073	47	3	101.1 (33.8- 302.2)
10- 19	6,709,271	1,464	44	1	104.2 (15.0- 723.5)
20- 29	7,454,809	3,521	42	1	50.4 (7.3- 349.7)
30- 39	7,264,770	6,322	41	2	56.1 (14.5- 216.6)
<20	13,100,862	5,537	47	5*	251.7 (109.9- 576.7)
<40	27,820,441	15,378	47	8*	307.9 (163.9- 579.0)

*As noted in the text above, one patient is known to have died before the age of 20 years but the exact age at death could not be established.

In total, the study identified 36 cases where the diagnosis of Pallister-Killian syndrome had been made in the antenatal period. It also identified two intra-uterine deaths, one stillbirth and eleven terminations of pregnancy for fetal abnormalities where Pallister-Killian syndrome was later diagnosed. This data, along with the data from the live births is summarised by year of delivery in Figure 17 below.

Figure 17: Summary of total cases identified



The sex of the patient was known for the study participants, those for whom death certificates were available and those diagnosed in our own laboratory. This data was also provided for some of the cases (both live births and terminations of pregnancy) identified by the cytogenetics laboratories, either by the laboratories themselves or by

the Medical Research Information Service. Overall, the sex was known in 55 cases. Of these, 39 were male and 16 were female. The chi-squared value for the difference between these values and the total population values is 9.70 when the whole population data is used and 7.98 when the population data for those aged 31 years or less is used. These values correspond to probability value of 0.002 and 0.005 respectively, both of which are statistically significant.

4c: Pregnancy And Delivery

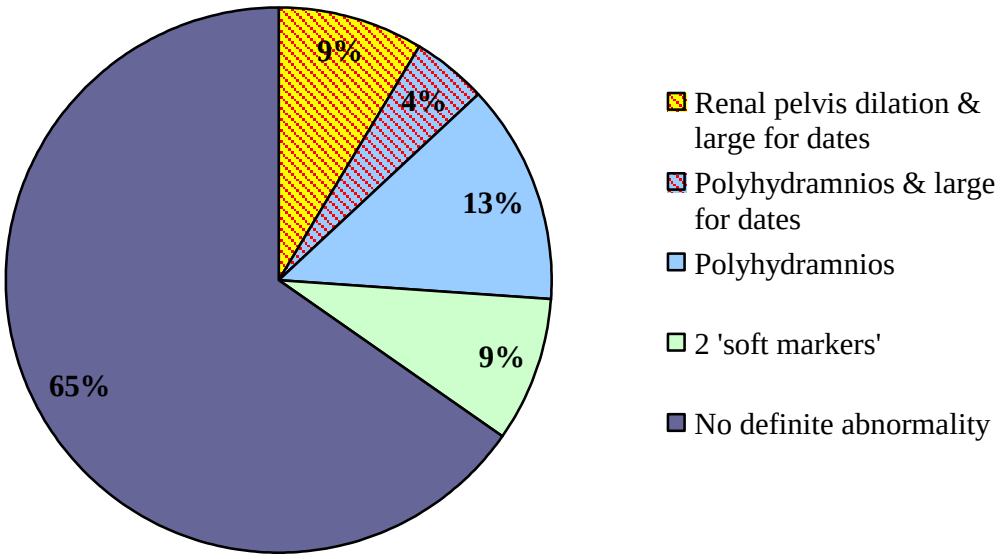
None of 23 pregnancies involving a baby with Pallister-Killian syndrome in the study had been achieved with the help of assisted reproductive technology. There was no significant history of miscarriages, with only three of the 23 mothers (13.0%) having a single first trimester miscarriage either before the patient's birth or subsequently. Equally, there was no family history of chromosomal disorders in the siblings of any of the recruited patients. This was the mother's first pregnancy to continue into the second trimester in six cases (26.1%), the second in nine cases (39.1%), third in five cases (21.7%) and fourth in three cases (13.0%).

Decreased or late fetal movements were reported in eight cases (34.8%), although in some cases this was a retrospective comparison with the patient's younger siblings. Ultrasound scans had been performed at least once during each of the pregnancies. Definite polyhydramnios was present in four cases (17.4%) and necessitated delivery at 35 weeks' gestation in two of these. In another six cases (26.1%), polyhydramnios or an excessive amount of amniotic fluid was mentioned at some point during the pregnancy or at delivery but no formal diagnosis was made so these cannot be counted as detectable fetal abnormalities. There was never any suggestion of either polyhydramnios or oligohydramnios in the other 13 pregnancies (56.5%), one of which was terminated at 18 weeks' gestation.

Three patients (13%) were noted to be large for dates, two of whom had mild renal pelvis dilation (8.7% of total group), while the other had definite polyhydramnios. Small stomachs were noted in two fetuses (8.7%), both of whom had other soft markers for chromosomal abnormalities. One had cranial ventricle measurements at the upper end of the normal range and karyotyping was therefore performed on an amniocentesis sample and the diagnosis of Pallister-Killian syndrome made. The other had areas of increased echogenicity in the bowel but a normal karyotype had already been obtained from a chorionic villus sample performed due to an increased nuchal translucency. The ultrasound scans in the other 15 cases (65.2%) were normal and no significant congenital anomalies were detected antenatally in any of these 23 patients.

A summary of the fetal ultrasound abnormalities detected in the study group is shown in Figure 18.

Figure 18: Antenatal ultrasound scan abnormalities seen in the study group



In those recruited to the study, invasive testing was carried out in five of the 23 pregnancies. In two cases, fetal anomalies had been detected, as described above. In the other three cases, karyotyping was carried out on either a chorionic villus sample or an amniocentesis sample due to increased maternal age. Two out of the three amniocentesis results (66.6%) showed that the baby had Pallister-Killian syndrome but both chorionic villus samples in those recruited to this study showed a normal karyotype.

Combining the data from the study participants with that obtained from the cytogenetics laboratories shows that Pallister-Killian syndrome has been detected in at least nine chorionic villus samples and 27 amniocentesis samples in Great Britain. Pallister-Killian syndrome has also been diagnosed in 11 fetuses terminated because of fetal structural abnormalities, in two cases of intra-uterine death and in one stillbirth. Two of the cases detected after termination of pregnancy had previously had chorionic villus samples with normal results. When these two cases are added to the two study participants with normal karyotypes on chorionic villus sampling, this means that four

out of 13 chorionic villus samples (30.8%) performed when the fetus had Pallister-Killian syndrome gave false negative results. For amniocentesis testing, one out of 28 (3.6%) gave false negative results. When only recent data (2005 onwards) is included, two out of five reported chorionic villus samples (40.0%) gave normal results, while none of the 11 amniocentesis samples did. The chi-squared values for the differences between the results of the chorionic villus samples and amniocentesis samples are 3.81 for all samples and 1.89 for the recent samples. These equate to probability values of 0.051 and 0.17 respectively and are therefore not statistically significant.

One of the patients was born significantly prematurely at 29 weeks gestation and three were slightly premature (35 weeks gestation). The others were all delivered at term (between 38 and 41 weeks gestation). The chi-squared value for the difference between the rates of premature delivery (less than 37 weeks gestation) seen in the study group and in the population is 3.51, which equates to a probability value of 0.06 and is therefore not statistically significant.

The birth weights, head circumferences and lengths of the study group are reported in 4f: Growth And Development.

4d: Congenital Anomalies In Terminated Pregnancies

Only two of the study group had an antenatal diagnosis of Pallister-Killian syndrome. One of these resulted in a termination of pregnancy and the other in a live birth. The vast majority of the prenatal diagnoses (34/36; 94.4%) were identified from laboratory data. In all of those where follow-up data was available, a termination of pregnancy took place. Data supplied by the cytogenetics laboratories also identified all of the post mortem diagnoses made following termination of pregnancy (11/11), all of the intra-uterine deaths (2/2) and the single stillbirth.

The intra-uterine deaths had occurred at 36 and 38 weeks gestation but the gestation of the stillbirth was not recorded. Two of this group had unspecified congenital anomalies and the other had ventriculomegaly, along with an increased nuchal screening risk.

The commonest congenital malformation reported was a diaphragmatic hernia. This was present in 16 of the 45 cases (35.6%) identified by the cytogenetics laboratories as having either a prenatal diagnosis or a post mortem diagnosis after termination of pregnancy. In six of these cases this was the only fetal structural malformation reported. Five others had multiple congenital abnormalities, which in one case included a cardiac defect. Another four had the presence of at least one soft marker, such as short femurs, talipes or ventriculomegaly documented.

Cardiac defects were reported in four cases, including two with multiple congenital abnormalities, one of whom had a diaphragmatic hernia.

Polyhydramnios was seen in three cases and a diaphragmatic hernia was present in one of them. It was an isolated anomaly in one, while the other had an absent hand and ventriculomegaly. Three other cases had soft markers, without any known structural malformations. In the other seven cases with fetal abnormalities, the information supplied to the laboratory made it clear that fetal abnormalities had been detected but did not elaborate.

The congenital abnormalities identified in this group are shown in Table 12 below.

Table 12: Congenital malformations in terminations of pregnancy

Neurological and cranial malformations
Ventriculomegaly (9 cases)
Absent vermis
Enlarged cysterna magna
Small cerebellum
Pierre-Robin sequence
Thoracic and abdominal malformations
Atrial isomerism
Ventricular septal defect
Diaphragmatic hernia (16 cases)
Thoracic kyphosis
Short ribs
Anal atresia
Hydronephrosis (2 cases)
Two vessel cord
Limb malformations
Absent hand
Clenched fists
Polydactyly (2 cases)
Digital defects
Short long bones (8 cases)
Rocker-bottom feet
Talipes (4 cases)
Other features
Polyhydramnios (3 cases)
Abnormal nuchal and/ or serum screening (16 cases)

A further 13 of the 34 antenatal diagnoses (38.2%) reported by the cytogenetics laboratories were made in the absence of any known fetal anomalies. Including those in the study group, this represents 14 of the total 35 terminations of pregnancy (42.9%). These procedures were carried out due to increased maternal age (6 cases; 17.1%), a raised serum screening result (3 cases; 8.6%), increased nuchal translucency (3 cases; 8.6%) or in a pregnancy at risk of a different disorder (1 case; 2.9%).

Including those with detected fetal abnormalities, 12 cases (34.3%) had an increased nuchal fold, three (8.3%) had a raised risk from serum screening and one (2.9%) had a high risk from the combined nuchal and serum findings. Overall, 16 of the 36 antenatal

diagnoses (44.4%) were made in the presence of an increased risk demonstrated by nuchal and/ or serum screening.

4e: Congenital And Skeletal Anomalies In Study Group

None of those recruited to the study had a diaphragmatic hernia or imperforate anus but two were known to have an anteriorly placed anus. One child had an obvious cleft palate at birth and another was later found to have a sub-mucous cleft palate along with a bifid uvula. None of the study group had a cleft lip.

Hydronephrosis had been detected antenatally in one child and resolved by the age of six months. It was also detected postnatally in a second patient. A further 11 had undergone renal ultrasound scans with no congenital abnormalities detected, although one of these had vesico-ureteric reflux. Overall, renal imaging had been performed in 13 of the 22 patients (59.1%).

Twenty of those with Pallister-Killian syndrome had an echocardiogram performed at some point and 16 (80%) of these were normal. Both of the children who had never had an echocardiogram were of school age so any significant congenital cardiac pathology should have been apparent by that stage. One child had an atrial septal defect that closed spontaneously by three months of age. The other three had valvular abnormalities, including pulmonary stenosis, mild mitral incompetence with some thickening of the valve and a dysplastic pulmonary valve. One of them, who had been born prematurely, had also had a persistent ductus arteriosus.

Inguinal hernias were reported in two boys (9% of patients and 14.3% of males). Both of these children had cryptorchidism, as did another two, so this occurred in 28.6% of boys. Umbilical hernias were present in two infants and one 11 year-old and had resolved in two other children.

Spina bifida occulta had been detected in one patient, although few of the others had ever had spinal X-rays. Scoliosis had been detected in six (27.3%) of the patients, between the first year of life and 14 years of age. All of these patients had significant hypotonia in the neonatal period. Four (66.7%) of them had remained floppy and were unable to sit without support, while the other two were able to walk independently. Underlying vertebral anomalies had been ruled out in two cases. Congenital dislocation of the hip was present in three cases, with two being bilateral and one being unilateral.

All of these children were in the significantly hypotonic group of neonates and one had also been in the breech position.

Twelve of the patients had previously had chest X-rays performed and it was possible to determine the number of ribs present in eight of these cases. Six of them (75%) had 12 pairs of ribs but two of them (25%) only had 11 pairs. In the other four cases where parents believed that the patient had had a chest X-ray, the relevant radiology department was either unable to find the X-ray or did not respond to the request to do so.

Other minor skeletal abnormalities seen included a patient with one triphalangeal thumb and another with unilateral arrested post-axial polydactyly.

The anterior fontanelle closed at the normal time (before 12 months of age) in six cases and was of normal size in the four month-old baby (31.8% combined). In another three cases (13.6%), the parents did not know when the fontanelle had closed but had never been told that it was delayed. One baby still had a large anterior fontanelle at 11 months of age and closure had been delayed beyond one year of age in eleven other cases (54.5% in total), with the fontanelle not closing until two or three years of age in five of these patients (22.7% of total group).

Eruption of the teeth was delayed in 12 cases (57.1%), with the first tooth emerging between 11 and 18 months of age in 11 cases and just before the child's third birthday in the other case. The teeth had emerged at the appropriate time in the other nine cases (42.9%) who were old enough to have teeth at the time of the study visit.

Bone age calculations had only been performed in one of the patients seen. His bone age was significantly delayed at approximately 15 months, when he was four years old.

4f: Growth And Development

Pre-natal Growth

Birth weights were available for all patients but the birth head circumference was only available for 14 patients (63.7%) and the birth length for seven patients (31.8%). In most cases, the actual measurements were recorded but one child's birth length and four children's birth head circumferences could only be found as plotted points on the patient's growth chart.

The birth weights of all of those in the study were above the 0.4th centile and below the 99.6th centile on the 1996 UK cross-sectional growth charts and therefore fall within 2.67 standard deviations of the mean. Seven of them had birth weights below the 50th centile, while 14 had birth weights above this line and one had a birth weight that fitted exactly onto the line. The chi-squared value for the difference between these values and the expected population value (50% below the 50th centile line) is 2.00, which is not statistically significant (p= 0.16). The birth weights are shown in comparison to the growth chart centiles in Figure 19. Figure 20 then shows these and the birth lengths and head circumferences, in comparison to the expected population curve.

Figure 19: Birth weights in study group compared to normal distribution

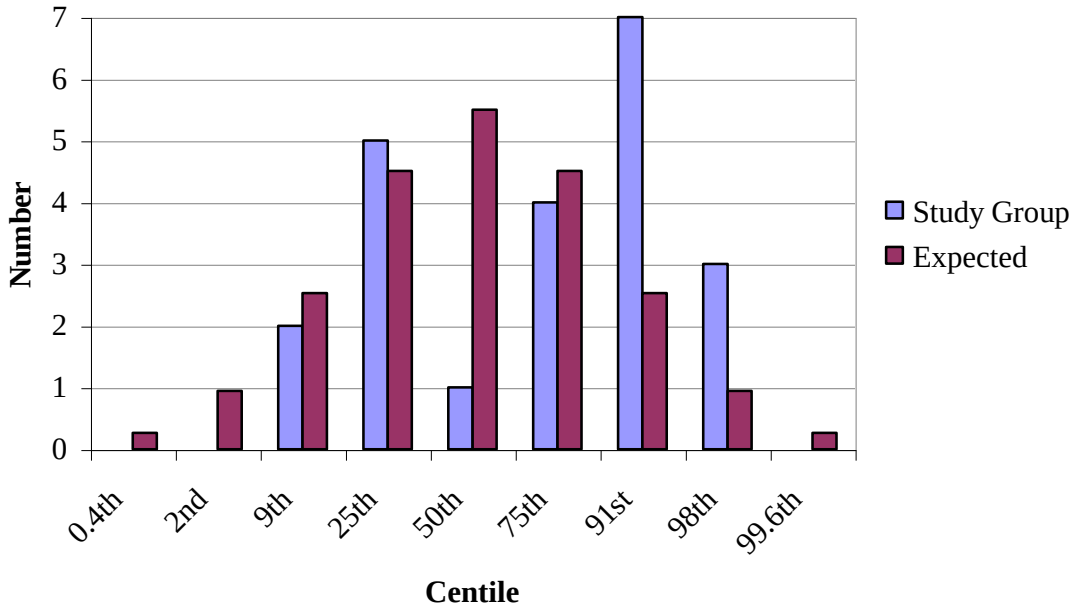
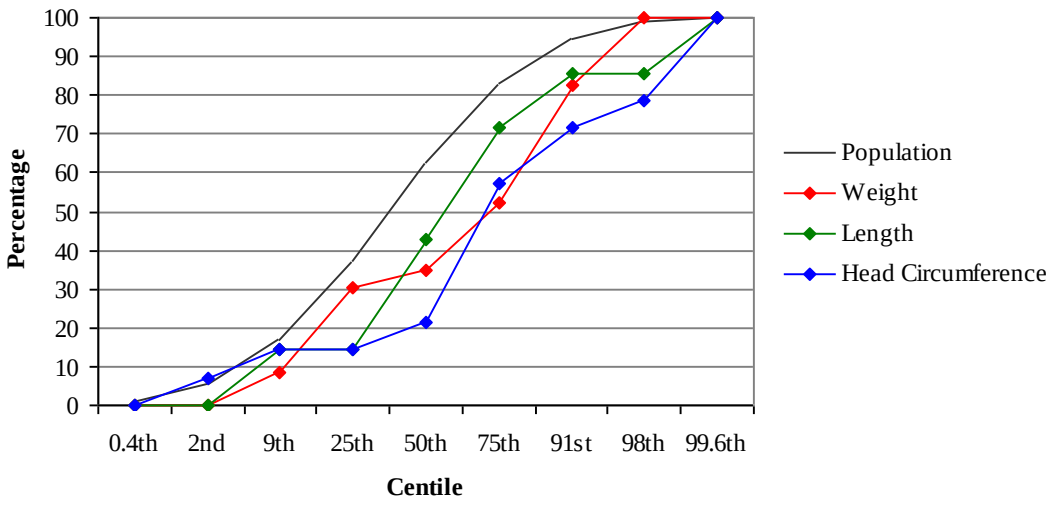


Figure 20: Birth measurements in study group compared to normal distribution

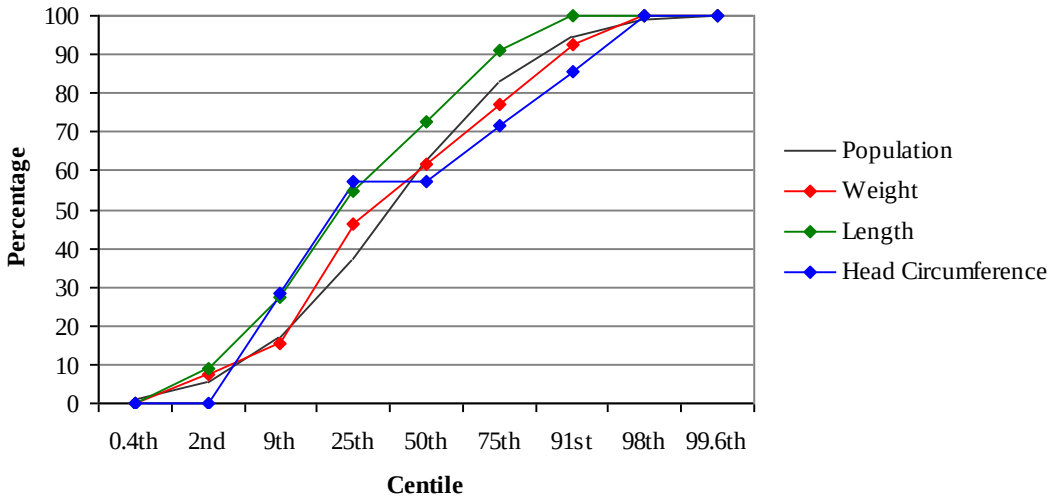


Postnatal Growth

Records of previous measurements, especially head circumferences, were often unavailable. Fourteen (36.8%) of the 38 childhood weight records and 16 (47.1%) of the 34 height records were available only from plotted points on the growth chart, rather than as actual measurements.

Twenty patients were aged more than 18 months (with the youngest in this group being 22 months old) and were therefore eligible for the growth parameters analysis at approximately two years. Of these, weight was recorded for 13 (65.0%), height for nine (45.0%) and head circumference for seven (35.0%). These are shown in Figure 21 in comparison to the population growth curve.

Figure 21: Growth measurements at approximately two years



For the 13 patients aged at least four years, a height and weight at approximately five years was available in eight cases (61.5%) and these are shown in Figure 22. None of the families had access to head circumference data beyond two years of age so the only data comes from the study measurements. Three children were seen at four or five years old and their head circumferences approximated the 2nd, 75th and 98th centiles. Due to the small numbers, this data has not been plotted in the graph (Figure 22). For those aged at least 10 years, recent growth parameters, including head circumferences measured during the study meeting, are shown in Figure 23.

Figure 22: Height and weight at approximately five years

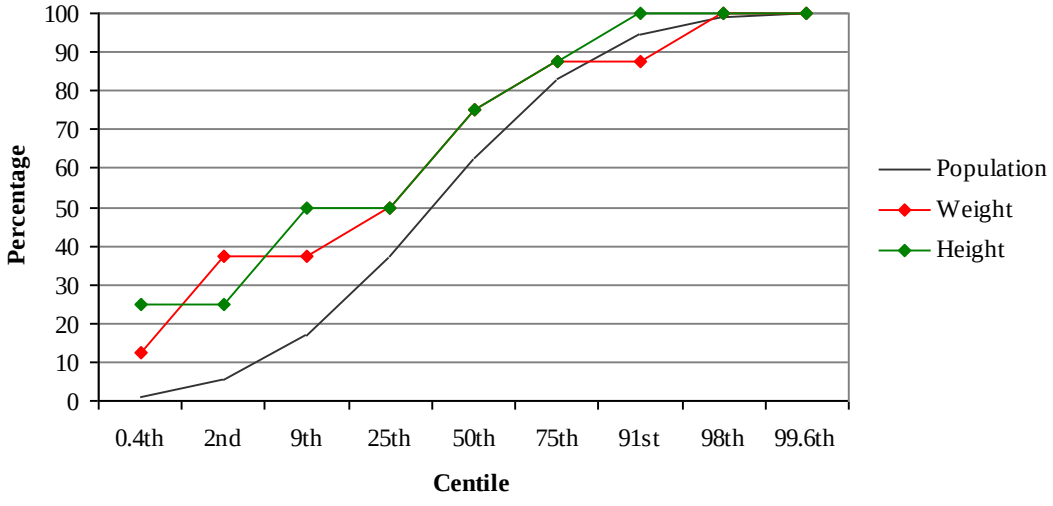
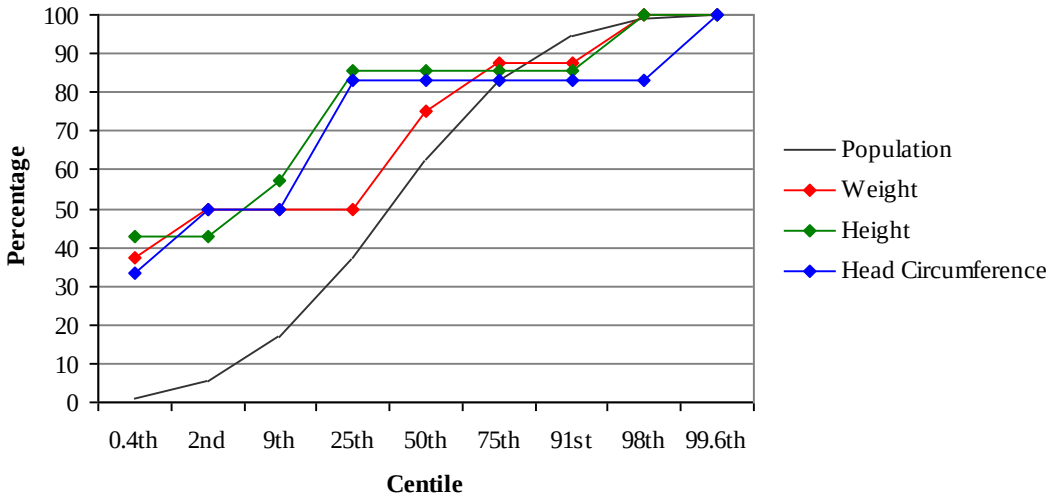


Figure 23: Growth measurements from at least 10 years of age



Development

Developmental delay of some degree was apparent in all of the patients, except for the four month-old baby. The degree of developmental delay was very variable, with three children coping in mainstream schooling. The results of the Denver II developmental screening test are shown for each patient in Figure 25 and Figure 26.

Nine of the patients had learnt to walk and this occurred between the ages of 16 months and eight years. At 18 months old, the upper end of the normal range for starting to walk, only one out of 20 children (5.0%) had begun walking. By the third birthday, the figure was five out of 17 children (29.4%) and by the fourth birthday seven out of 13 (53.8%). Three children aged 10 or 11 years were still unable to walk. Eight of the 11 children (72.7%) aged more than one year who were unable to walk were also unable to sit without support. This is shown graphically in Figure 24 below.

Figure 24: Number of patients walking by each birthday

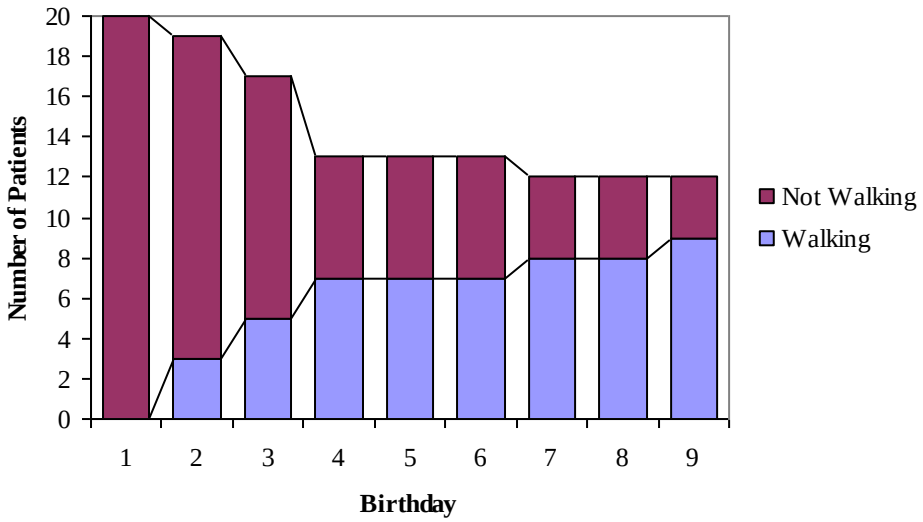


Figure 25: Developmental age for those under five years

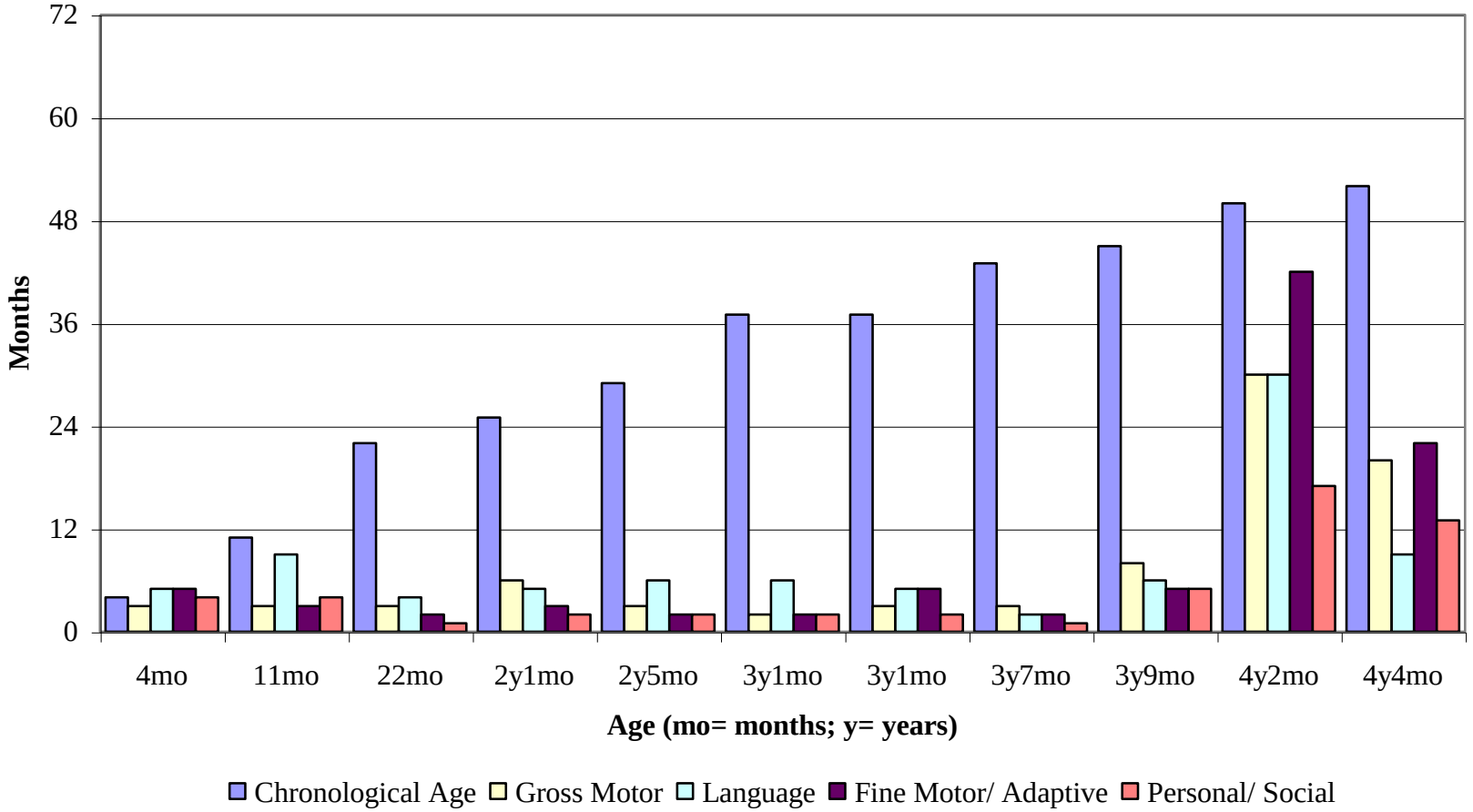
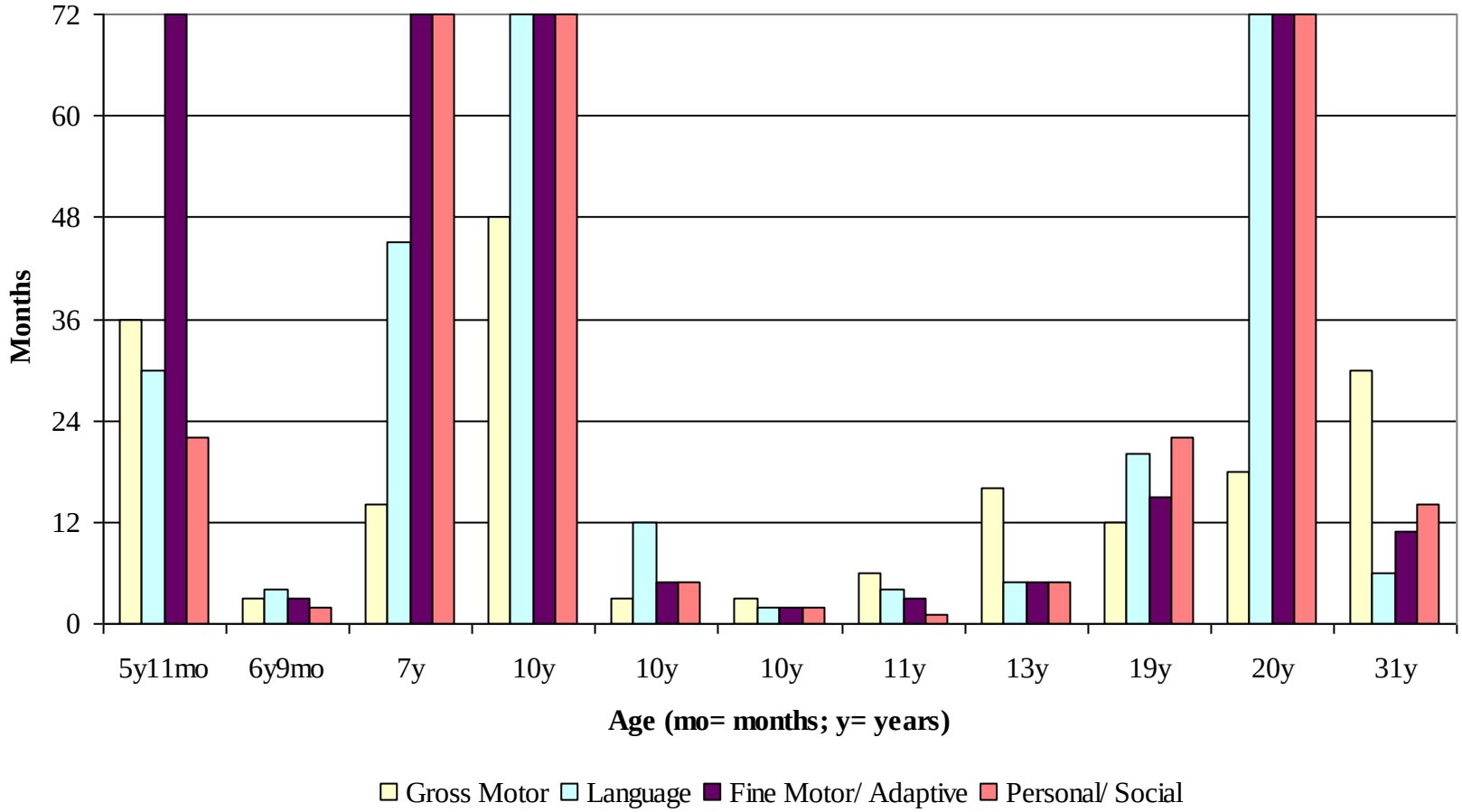


Figure 26: Developmental age for those over five years



4g: Neurology, Hearing And Vision

Significant hypotonia was present in most Pallister-Killian patients in the neonatal period (17/22; 77.3%). Mild hypotonia had been noted in three (13.6%) and the other two (9.1%) were thought to have had normal tone. At the time of examination, 11 patients (out of 21; 52.4%) had marked hypotonia, one had milder hypotonia (4.8%), one had significant spasticity (4.8%) and eight (38.1%) had normal tone. Of those who had significant neonatal hypotonia, 10 (58.8%) had remained floppy, one (5.9%) had milder hypotonia, one (5.9%) was hypertonic, four (23.5%) had normal tone and one (5.9%) had died. The three patients who had been mildly hypotonic as neonates all had normal tone on examination. One of the children reported to have normal tone in the neonatal period was significantly hypotonic on examination, while the other still had normal tone.

Significant feeding difficulties were present in six cases (27.3%), with one premature baby requiring a gastrostomy and two babies, including one said to have normal tone as a neonate, requiring nasogastric feeding. Mild feeding difficulties were noted in nine cases (40.9%) but one of these children had a cleft palate and his feeding difficulties were no greater than expected for babies with cleft palates. If he is excluded, this means that 14 out of 21 (66.7%) had some degree of neonatal feeding difficulties.

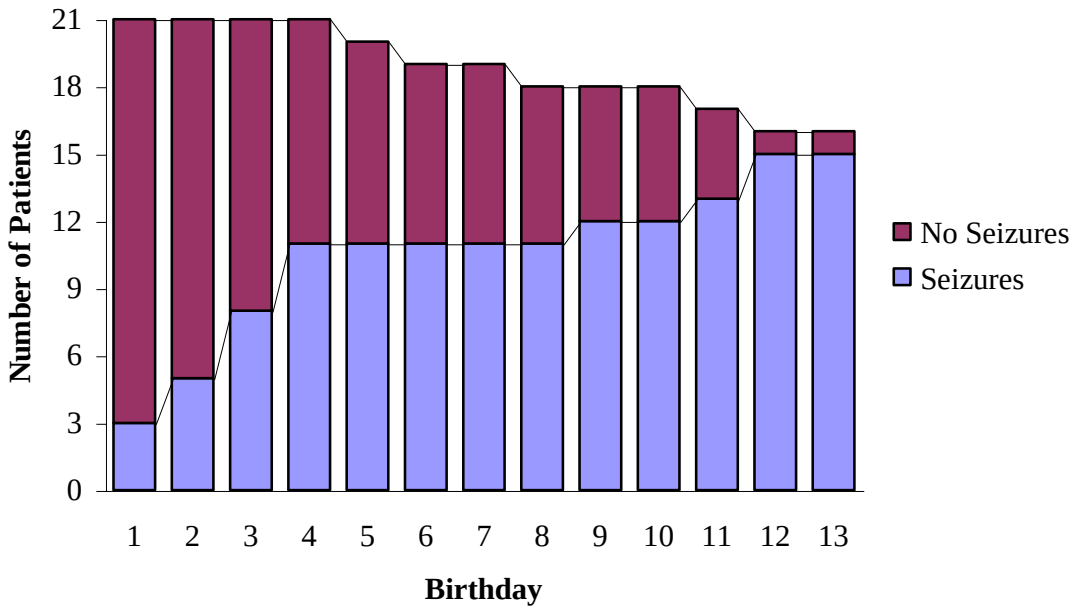
Seizures were present in 15 of the patients (68.2%) at the time of review. A further child, the oldest without clinical seizures, had purely sub-clinical seizures identified on an electroencephalogram recording, meaning that 16 (72.7%) had had some form of epileptiform activity recorded. The age of onset of clinical seizures was either between six months and the fourth birthday or aged eight to 11 years. Only 10 children (out of 21; 47.6%) reached, or would reach, their fourth birthdays without having seizures, as shown in Table 13 and Figure 27 below. The majority of the patients with clinical seizures had myoclonic seizures (9/15; 60.0%; 40.9% of total study group), although in one these had evolved into complex partial, absence and occasional generalised tonic-clonic seizures during puberty. A further four patients (26.7% of those with clinical seizures; 18.2% of study group) had generalised tonic-clonic seizures. One of these children and two of those with myoclonic seizures had absence seizures prior to the

development of the current seizure type developed. Late-onset infantile spasms, starting at two years of age and tonic seizures were each seen in one patient.

Table 13: Onset of clinical seizures by age

Birthday	Patients with seizures by age		Patients reaching age without seizures	
1st	3	(14.3%)	18	(85.7%)
2nd	5	(23.8%)	16	(76.2%)
3rd	8	(38.1%)	13	(61.9%)
4th	11	(52.4%)	10	(47.6%)
5th	11	(55.0%)	9	(45.0%)
6th	11	(57.9%)	8	(42.1%)
7th	11	(57.9%)	8	(42.1%)
8th	11	(61.1%)	7	(38.9%)
9th	12	(66.7%)	6	(33.3%)
10th	12	(66.7%)	6	(33.3%)
11th	13	(76.5%)	4	(23.5%)
12th	15	(93.8%)	1	(6.3%)
13th	15	(93.8%)	1	(6.3%)

Figure 27: Clinical seizures by age



Self-stimulatory behaviour such as rocking or head shaking was seen in 10 patients (45.5%). Six of these patients, along with two of the others (eight in total; 36.4%) had self-harming tendencies, mainly involving biting of hands. Autistic features, including

the need for routine and arranging objects were reported in six cases (27.3%). Five of the patients (22.7%) had episodes of hyperventilation, which were interspersed with breath holding in two cases. One of these children also suffered from sleep apnoeas.

Difficulties with sleeping were reported in half of the patients, with five being difficult to settle, five waking repeatedly during the night, in one case due to nocturnal seizures, and one waking early.

Most of the 22 patients with Pallister-Killian syndrome had some hearing difficulties. Seven (31.8%) of them had significant sensorineural hearing impairment and required hearing aids. In one of these cases, there was also a conductive component to the hearing impairment. Another one had definite conductive hearing impairment but an additional sensorineural component was being considered. A further nine had conductive hearing impairment, giving a total of 45.5% of the group having hearing impairment that was mainly conductive and 50.0% having hearing impairment with a conductive component. Three of them had required grommets. Normal hearing had been confirmed in only four cases (18.2%). A fifth patient was believed to have normal levels of hearing but had not had a formal audiology review to confirm this.

Similarly, only five (22.7%) of the patients had normal vision. Nine (40.9%) had significant abnormalities of the visual pathways, with five (22.7%) having nystagmus, three (13.6%) having optic nerve hypoplasia, one of whom also had macular hypoplasia and was registered blind, and one (4.5%) having retinal hypopigmentation. Each of these patients had additional visual abnormalities, including myopia, hypermetropia, astigmatisms and squints. The remaining nine patients (40.9%) had one or more of these later abnormalities. Retinal pigmentary abnormalities had been recorded in three patients (13.6%).

Cranial imaging had been performed in 15 cases (68.2%), with 12 patients having magnetic resonance imaging, two having had computerised axial tomography and one having had a cranial ultrasound scan as a baby. Reports for 14 of these investigations were available from the parents, their copies of letters or the hospital that had performed the investigation and nine (64.3%) of them gave essentially normal results. Abnormalities of the corpus callosum were reported in three cases (21.4%), with

agenesis reported in one case and mild deficiency reported in two. The one with agenesis of the corpus callosum also had a low brain volume with increased extra-axial cerebrospinal fluid space. One of those with thinning of the corpus callosum had delayed myelination and the other had mild ventriculomegaly. Another child had both delayed myelination and mild ventriculomegaly but a normal corpus callosum. The final abnormality found on cranial imaging was isolated mild ventriculomegaly in one patient. Overall, three out of the 14 had mild ventriculomegaly (21.4%) and two had delayed myelination (14.3%).

4h: Endocrine And Immune Features

Twelve of those with Pallister-Killian syndrome were believed to have more infections than other children of a similar age, although none had been shown to have any specific immunological deficiency. Three of them had a general increase in all types of infections but did not have severe infections. Another nine were prone to respiratory tract infections and four of them had required hospital admissions because of pneumonias. Three of them also had recurrent urinary tract infections. The other 10 patients were thought to have similar numbers and severities of infections to their siblings and contemporaries, although one who was generally healthy had recently had a liver abscess.

Four females were old enough to have been expected to have entered or completed puberty and this had occurred within the normal age ranges. Two males had delayed puberty, with one entering puberty at 16 years of age and a second not having reached this stage at the time of the visit, when he was 13 years of age. The other males were not old enough to expect the first stages of puberty to be apparent.

Nine of the patients (40.9%) were reported to have anhydrosis or hypohydrosis, with none of them having excessive sweating.

4i: Facial Dysmorphology

As babies, all the patients were reported to have had absence of hair in the fronto-temporal regions. This gradually improved with age but all nine of those examined at less than four years of age still had some bald patches in the fronto-temporal regions. In seven of them (77.8%), the hair covering the other areas of the scalp remained sparse, while the other two had thin hair that was within the normal spectrum. In the older patients, three (25%), including one adult, still had areas of complete alopecia and three (25%) had sparse hair within the previously bald areas. In a further three cases (25%) the previously bald areas of the scalp had an appropriate number of hairs but these would only grow to a few centimetres in length.

The anterior hairline was higher than expected in eight out of nine (88.9%) of those aged less than four years and, in one case (11.1%), the posterior hairline was similarly high. The posterior hairline was low in the single child under four years of age (11.1%) who had a normal anterior hairline. The anterior and posterior hairlines were also high in one of the four year-old children and one ten year-old showed a normal anterior hairline with a high posterior hairline. All of the other children aged at least four years had normal anterior and posterior hairlines, with the exception of the patchy alopecia mentioned above. Both of the living adults had low anterior hairlines with normal posterior hairlines but the photographs of the adult who had recently died clearly showed a high anterior hairline.

In just over half of the patients (11/21; 52.4%), abnormally coarse or wiry hair was noted. This occurred mainly in the fronto-temporal regions, where the hair had been absent in infancy. One patient (4.8%) also had unusual hair pigmentation, with distinct patches of darker hair present.

Eyebrows were sparse in four children aged three years or less (44.4% of that group and 19.0% of total group) and the eyelashes were also sparse in three of these patients (33.3% of those aged three years or less and 14.3% of total group). Thick eyebrows were noted in five of the older individuals (41.7% of those aged at least four years and 23.8% of total group), including one of the four-year olds. One of the patients with thick eyebrows (4.8% of total group) also had long eyelashes. Normal eyebrow volume

was present in 12 individuals (57.1%) and the eyebrow shape appeared normal in all cases.

The head shapes were normal in most cases (14; 66.6%). Trigoncephaly was present in one adult patient and plagiocephaly in one of the infants. Brachycephaly was present in two cases (9.5%) along with a flat occiput, which was present in isolation in a further three cases (23.8% in total). No abnormalities of the sutures were detected and the fontanelles have already been described in section 4e: Congenital And Skeletal Anomalies In Study Group.

Coarse facial features were apparent in 11 of the patients examined (52.4%). This was present in none of the five patients with a mild phenotype, four of the nine patients with a moderate phenotype (44.4%) and all of the seven patients with a severe phenotype. It was seen in only five of those aged less than ten years (5/14; 35.7% of this group) but present in six of the seven patients examined beyond this age (85.7% of this group). The youngest child with coarse facial features was only 22 months old and had a severe phenotype. One child with coarse facial features and a moderate phenotype was three years and nine months old but the other three were all aged more than 10 years.

Epicanthic folds were seen in four patients (19.0%), the oldest of whom was seven years of age. Mild bilateral ptosis was present in five patients (23.8%), with ages ranging from three years to adulthood. The palpebral fissures were upslanted in nine patients (42.9%), downslanted in one patient (4.8%) and normal in the other 11 cases (52.4%). Hypertelorism was present in 12 cases (57.1%). One patient (4.8%) had elongated palpebral fissures, while another 4 (19.0%) had short palpebral fissures. No abnormalities of the iris, pupil or sclera were noted in any of the patients.

Almost all of the patients had short noses (20/21; 95.2%), with anteverted nares in 10 (47.6%). The nasal bridge was depressed in all of the patients aged less than five years and in two of the others, who were aged six and 10 years. It was prominent in one of the adults and wide in the other. One patient (4.8%) had a low insertion of the columella but this was normal in the other patients.

The majority of the patients (15/21; 71.4%) had a long philtrum, which was smooth in four cases and prominent in five. In the patients with a normal length philtrum, it was smooth in two cases and prominent in one. Overall, seven patients (33.3%) had a prominent philtrum, five (23.8%) had a smooth philtrum and nine (42.9%) had normal philtral grooves. The variation in the philtrum did not appear to have any correlation with age.

An exaggerated cupid's bow to the mouth was common and was seen in 12 patients (57.1%), while three patients (14.3%) had a wide mouth. The vermilion of the upper lip was thin in five patients and thick in two. One of those with a thick upper lip vermilion also had a thick lower lip vermilion but this was also present in one who had a thin upper lip vermilion and in four who had a normal upper lip vermilion. Eversion of the lower lip vermilion was seen in seven patients (33.3%). This was mainly seen in the younger children (four out of five less than three years old) and the adults (both of them) but was also seen in one patient aged 10 years.

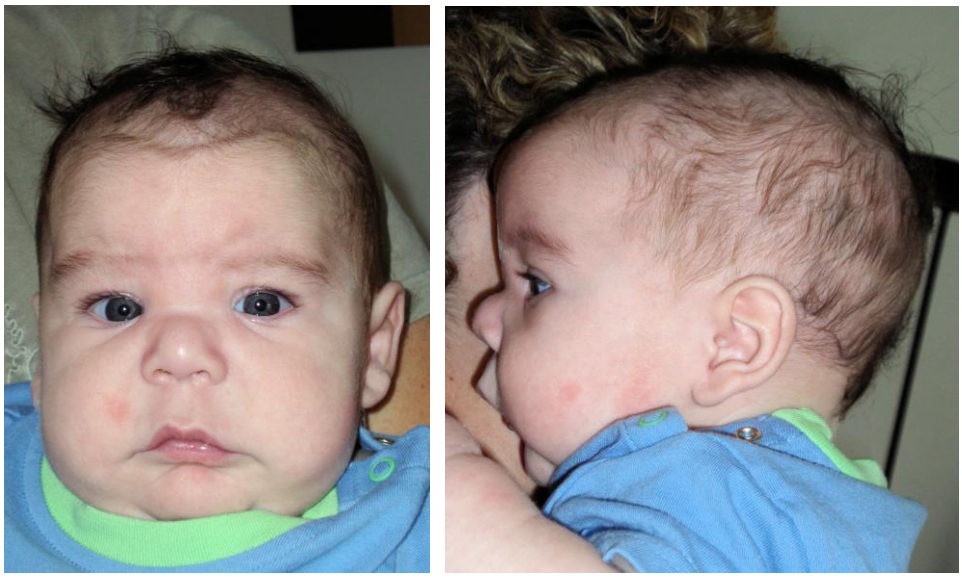
Seven patients (33.3%) had a palate that was higher than normal, including one with a repaired cleft, and one of them also had an unusually broad palate. The palate of the other patient with a repaired cleft appeared normal on clinical examination. Apart from microdontia in one patient (4.8%) and the delayed dentition that has previously been described, normal tooth formation was seen. A large, protuberant tongue was apparent in five cases (23.8%), ranging in age from a toddler to an adult.

The ears were often low-set (12/21; 57.1%) and posteriorly rotated (8/21; 38.1%). They appeared small in three cases (14.3%) and a variety of other dysmorphic ear features were noted in single patients. These included attached lobes, large lobes, large and attached lobes, uplifted lobes, unilateral absence of the anti-helix and prominence of the superior crus of the anti-helix.

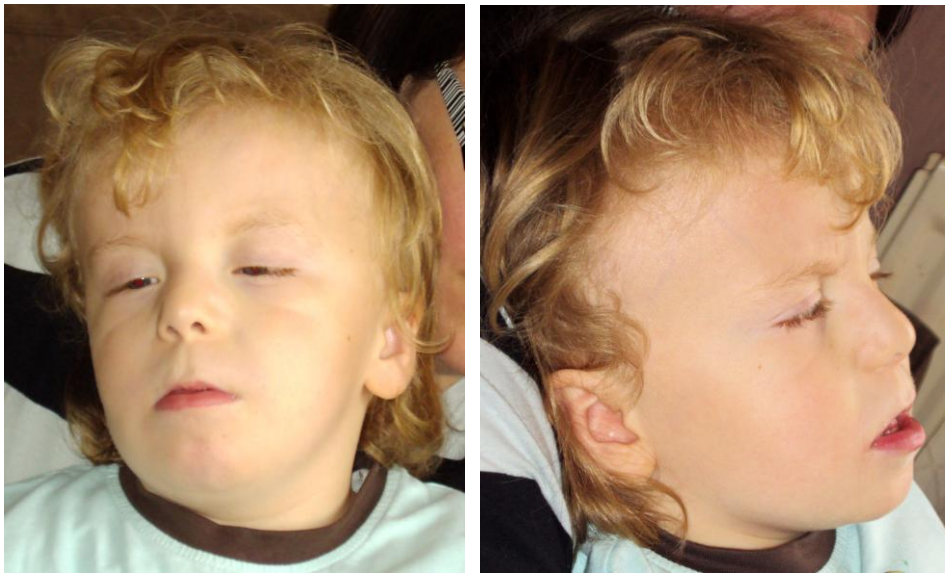
Two of the younger patients appeared to have short necks but none had redundant nuchal skin. Micrognathia was present in four children (19.0%), aged three years or less and prognathia was present in six patients (28.6%), all of whom were at least four years old. It is notable that all of the children aged less than two years had micrognathia, while both of the adults had prognathia.

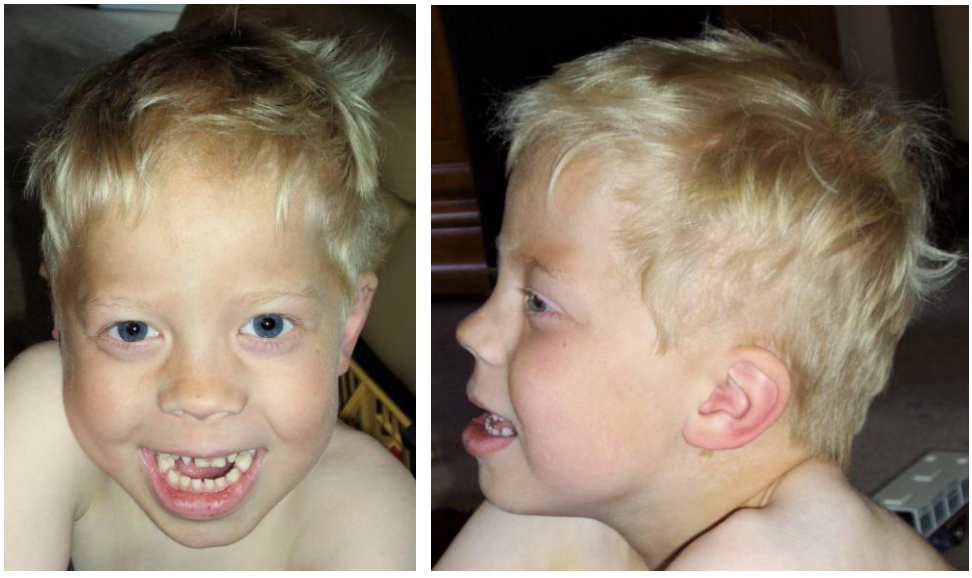
The dysmorphic features seemed to be more pronounced in those with a more severe phenotype and examples are shown in Figure 28, Figure 29 and Figure 30 below, with each group being displayed in ascending order of age.

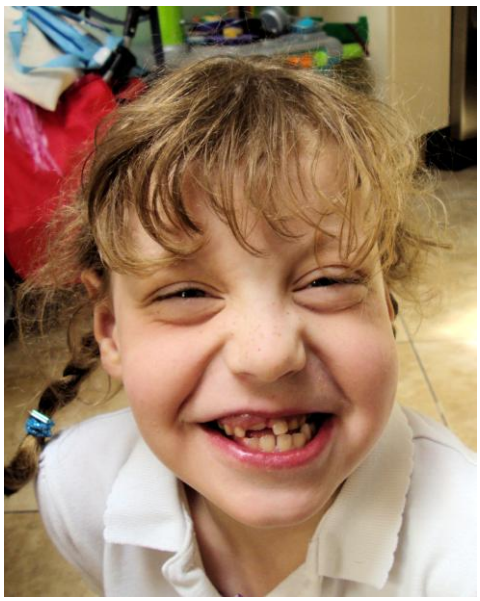
Figure 28: Facial features seen in those with milder phenotypes

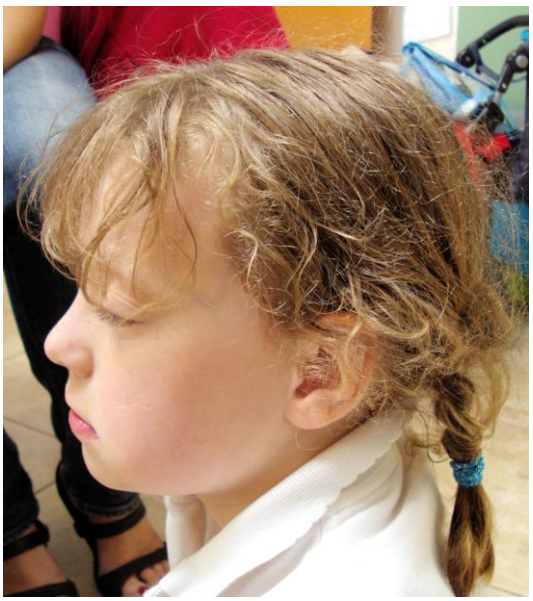


Note hypertelorism, mild epicanthic folds, long philtrum, and exaggerated cupid's bow.

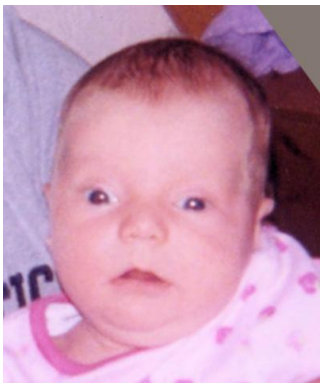


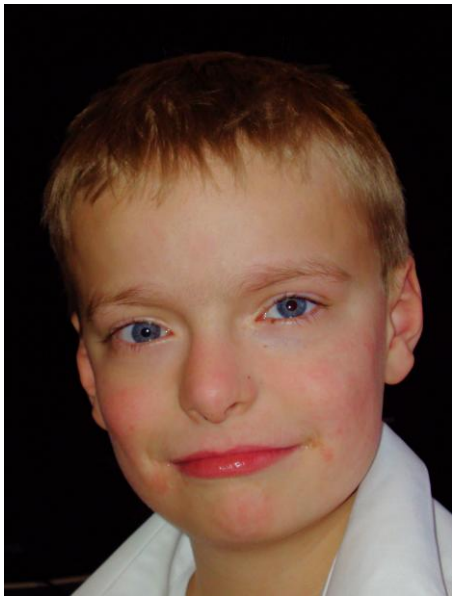






Patients above and below, respectively, as babies. Note the sparse hair in both that has improved with age.





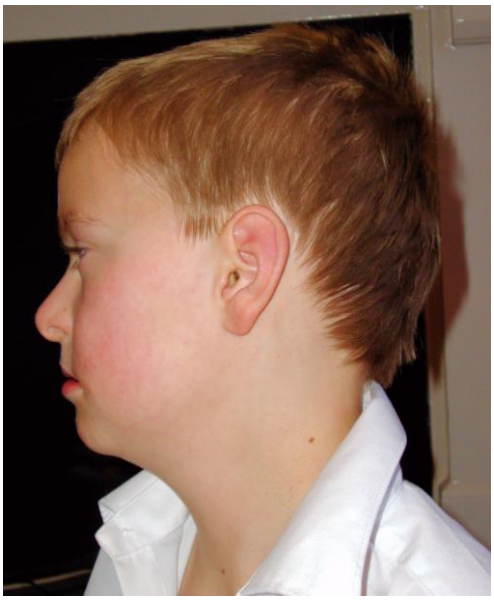
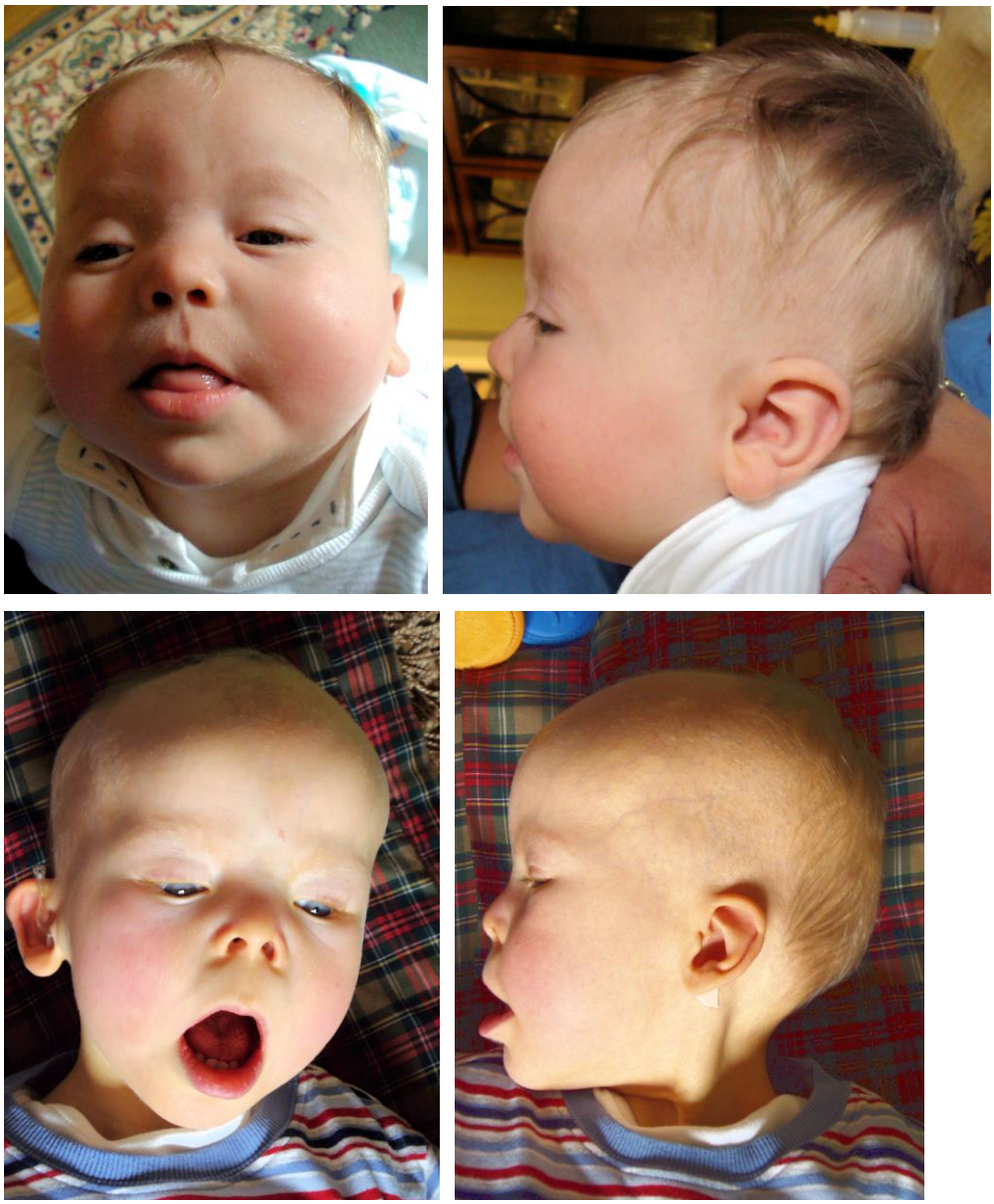
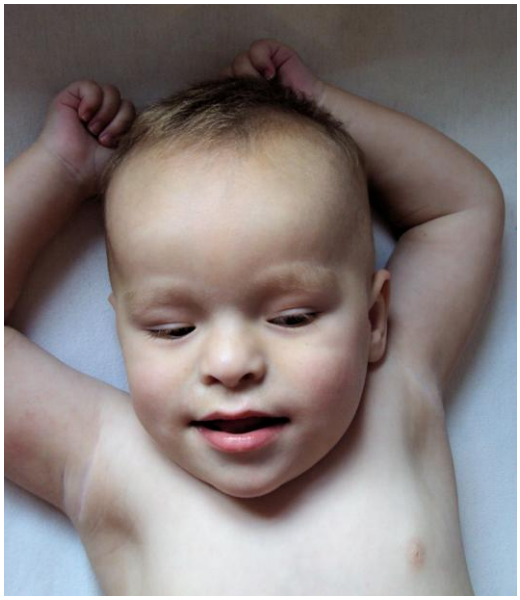
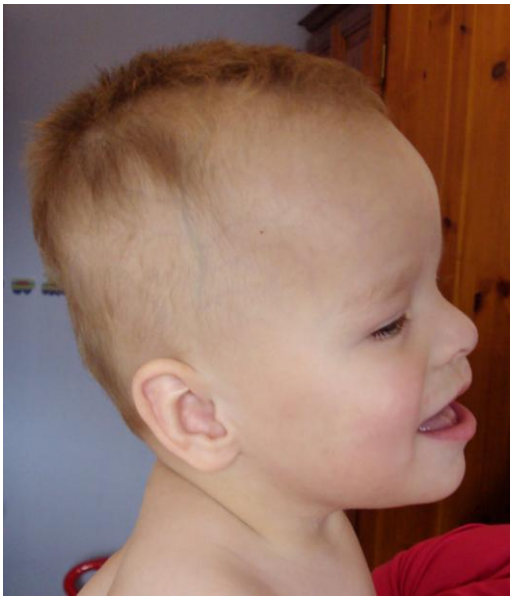
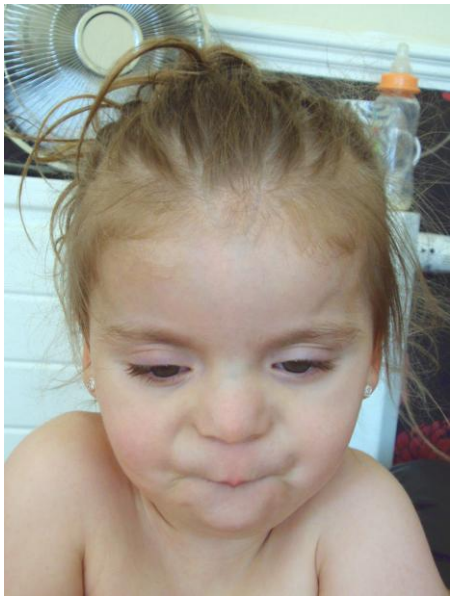
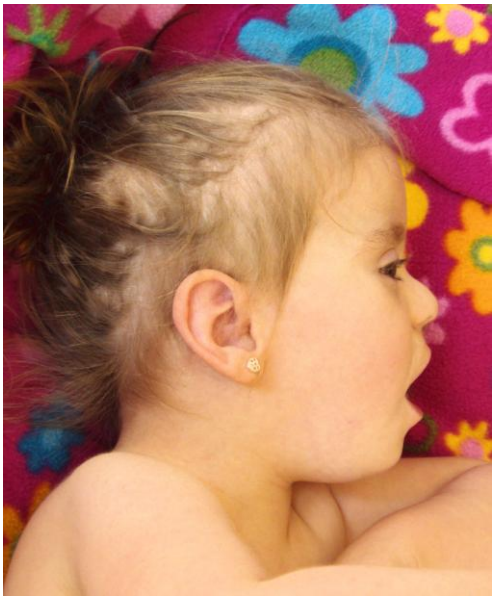
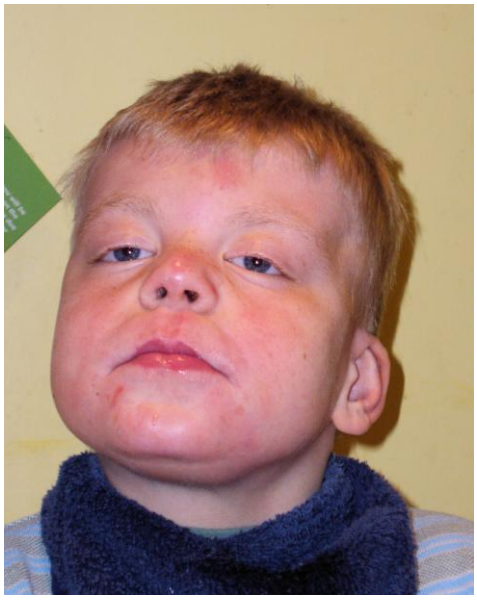
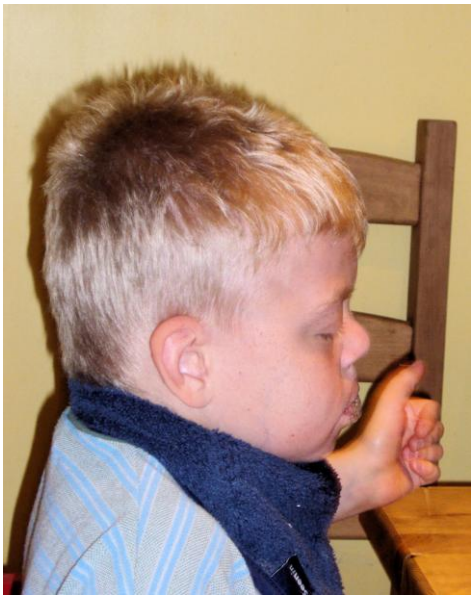
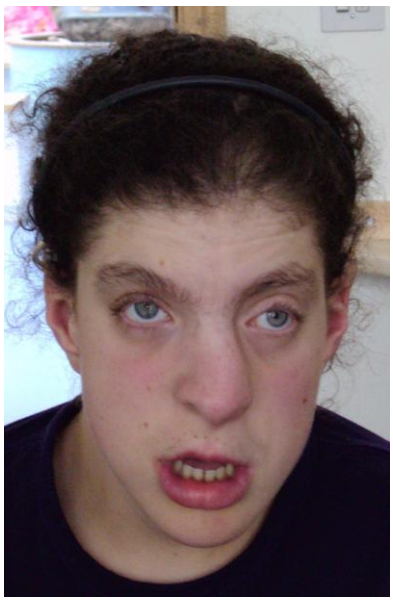
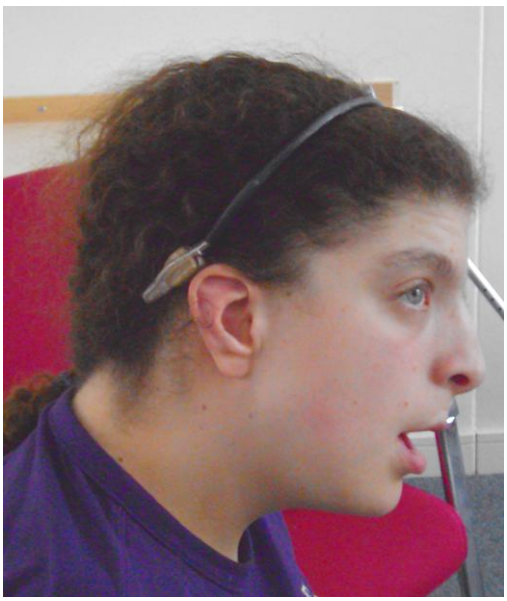
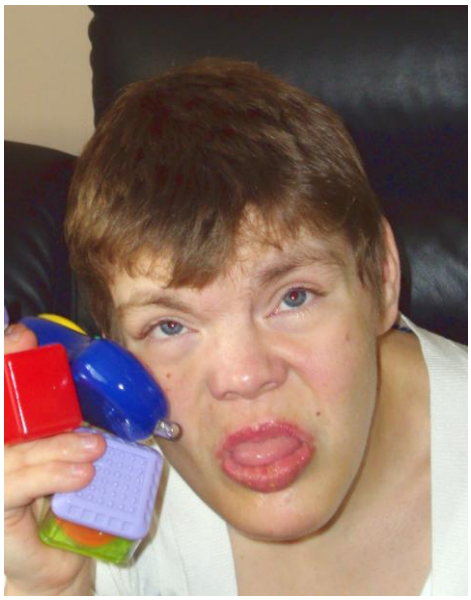
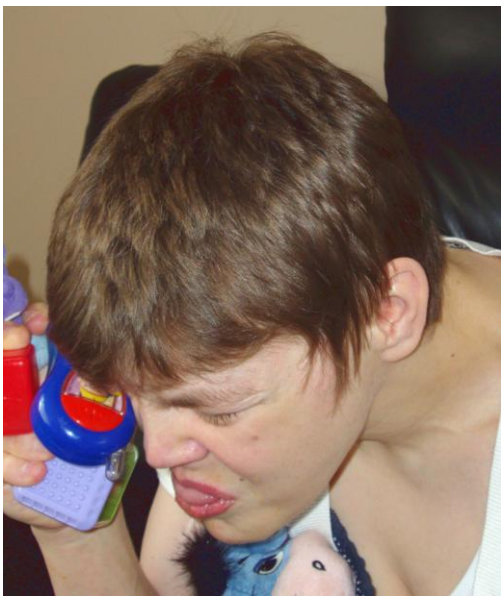


Figure 29: Facial features seen in those with moderate phenotypes



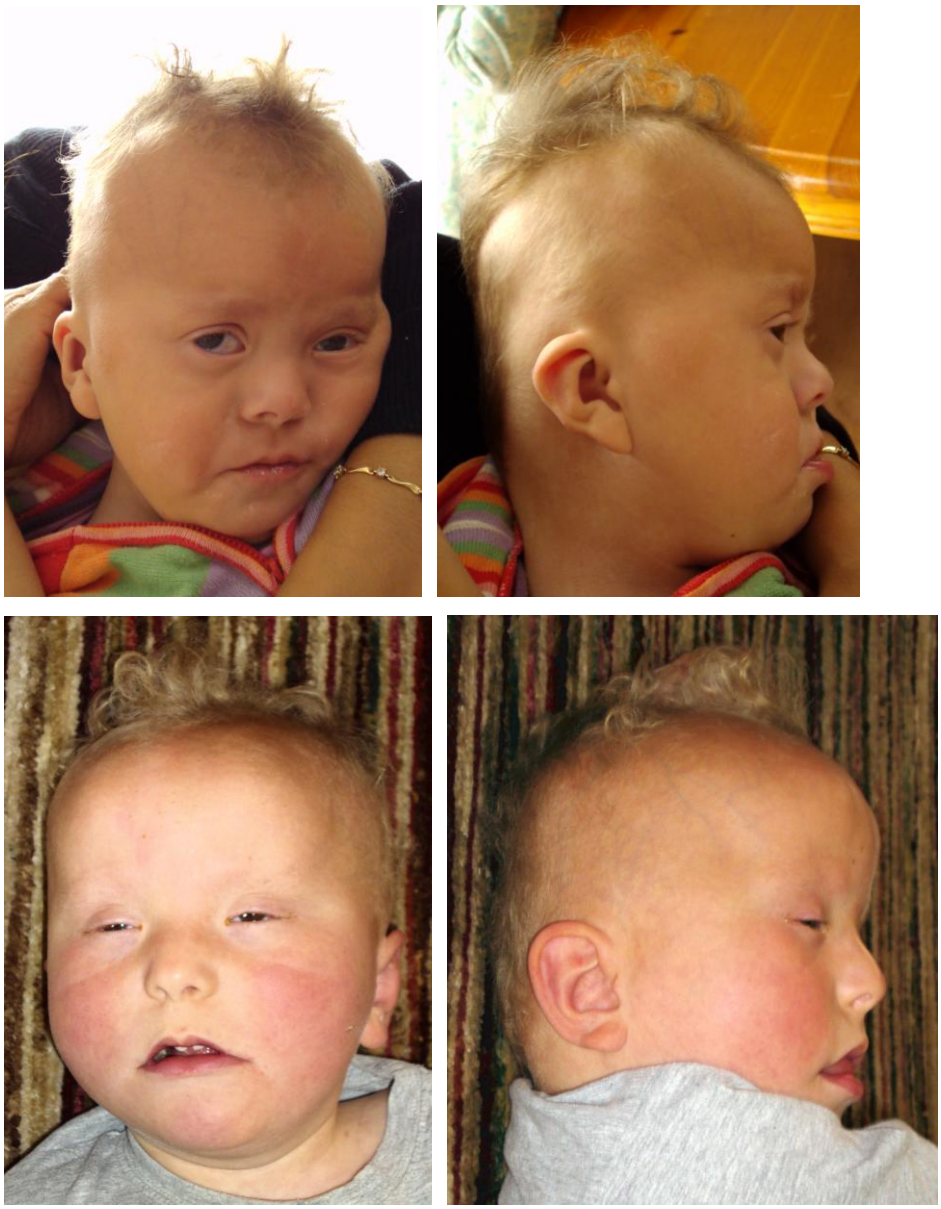
Note the sparse hair, low-set ears, short nose, anteverted nares and smooth philtrum.

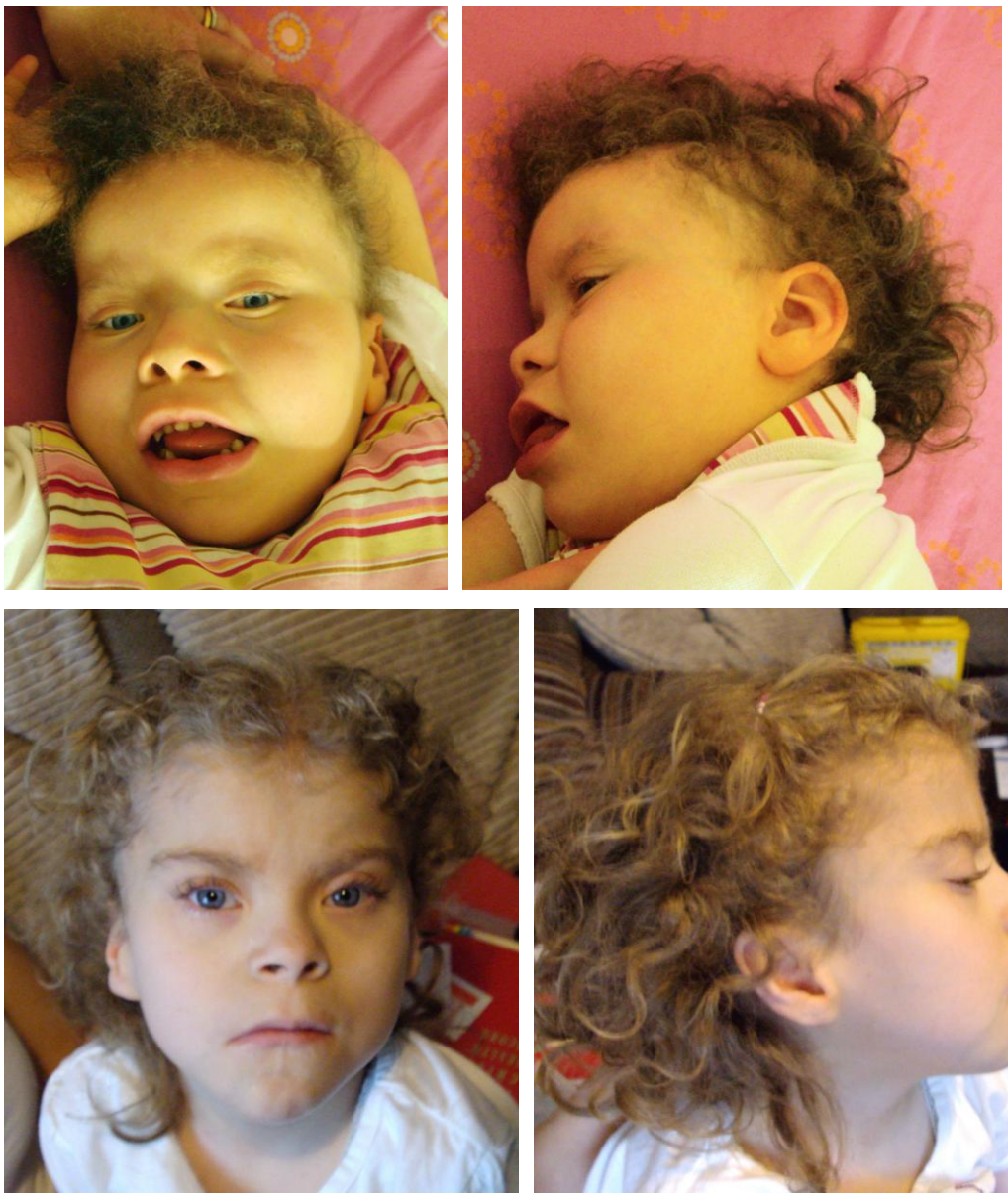





Note the coarse facial features, prognathia and protruding tongue.

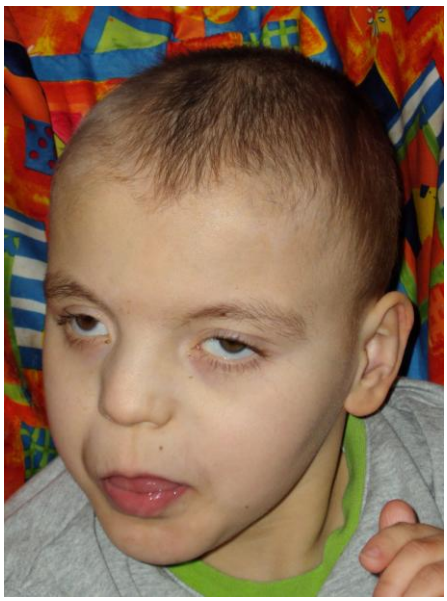
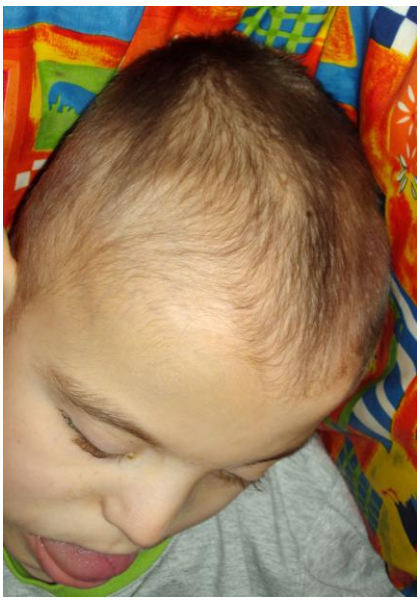
Figure 30: Facial features seen in those with severe phenotypes



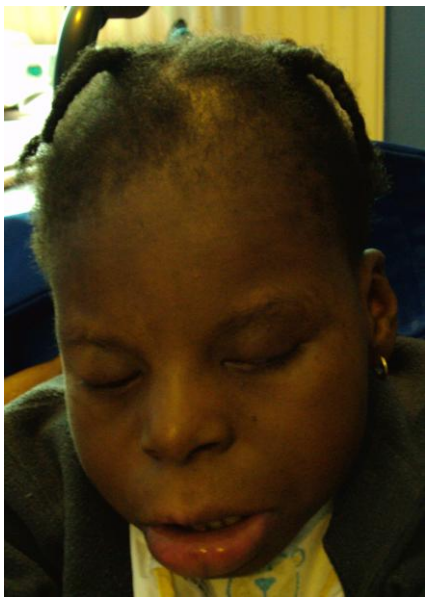
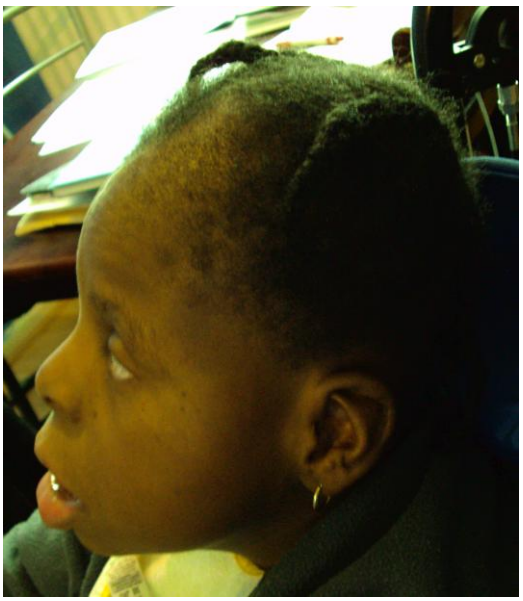
Note the sparse hair, eyelashes and eyebrows in both children, along with the depressed nasal bridges and short noses.



Note the coarse facial features, up-slanting palpebral fissures, prognathia and low-set, posteriorly-rotated ears.

Note the poor hair growth in the temporal regions, the hypertelorism, short nose, long, smooth philtrum and protruding tongue.

4j: Other Dysmorphology

The majority of the patients (14 out of 21; 66.7%) had some abnormalities of skin pigmentation. Three patients (14.3%) had hypopigmented lesions, one (4.8%) had hyperpigmented lesions and 10 (47.6%) had both. In five cases the pigmentary abnormalities followed Blaschko's lines (35.7% of those with pigmentary changes; 23.8% of total). Examples are shown in Figure 32 on page 121.

Accessory nipples were present in nine patients (42.6%). One child had one accessory nipple on the right and three accessory nipples on the left. Another four patients had a single accessory nipple bilaterally, with the remaining four patients having a unilateral accessory nipple.

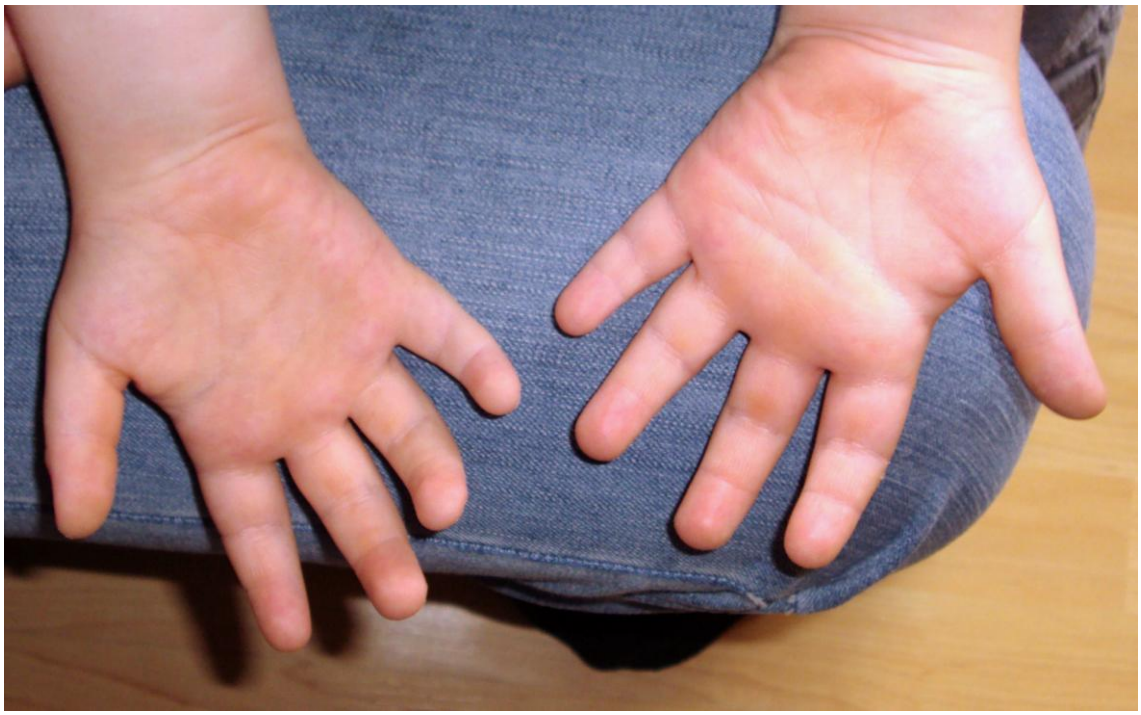
Shortening of the upper limbs was present in five individuals, with two of them having proportionate micromelia and the other three having rhizomelia. One of these patients had rhizomelic shortening of the both the upper and lower limbs. Mild limb oedema was present in one case (4.8%). Joint contractures were seen in two patients (9.5%), who were aged 11 and 13 years, and joint hypermobility in eight (38.1%), whose ages ranged from two to 10 years. An additional patient (4.8%), aged three years, had a mixed picture, with hypermobility of the small joints and contractures of the large joints.

Most of the patients (12/21; 57.1%) had short, broad palms and fingers (see Figure 31), while four (19.0%) had radial fifth finger clinodactyly. There were individual cases (4.8% for each) with one triphalangeal thumb, tapering fingers, broad fingertips and broad thumbs with short first metacarpals. Bilateral single transverse palmar creases were present in six individuals (28.6%), with unilateral single transverse palmar creases in four (19.0%) and bridged palmar creases in one case (4.8%). The palmar creases in the other 10 individuals (47.6%) were normal. One patient (4.8%) had longitudinal ridging of the finger nails and a different patient had cutaneous syndactyly of the second and third fingers on one hand and the second to fourth fingers on the other.

One individual (4.8%) had very short and narrow feet but the foot size in the others (20/21; 95.2%) was normal. In all cases (21/21; 100%), the plantar creases were normal

and there was no evidence of a sandal gap. Three of the patients had small fifth toenails and, in one case, the fourth toenails were also small. Mild cutaneous syndactyly between the second and third toes bilaterally was also detected in a single patient (4.8%).

Figure 31: Typical hands



Note the short, broad palms, short fingers and bilateral horizontal palmar creases. Subtle fifth finger clinodactyly may also be appreciated.

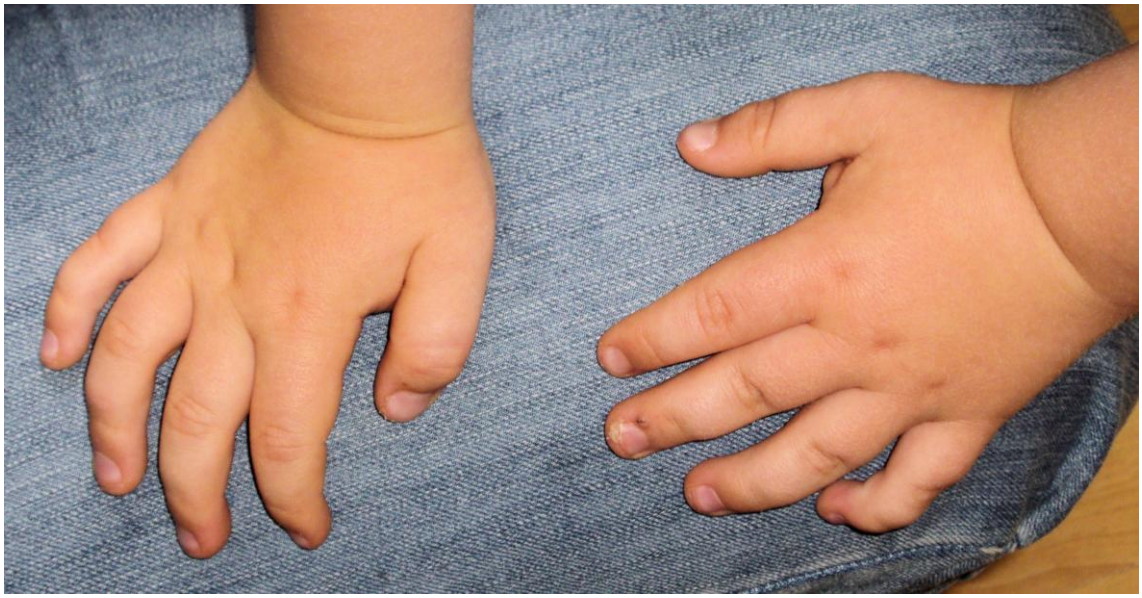
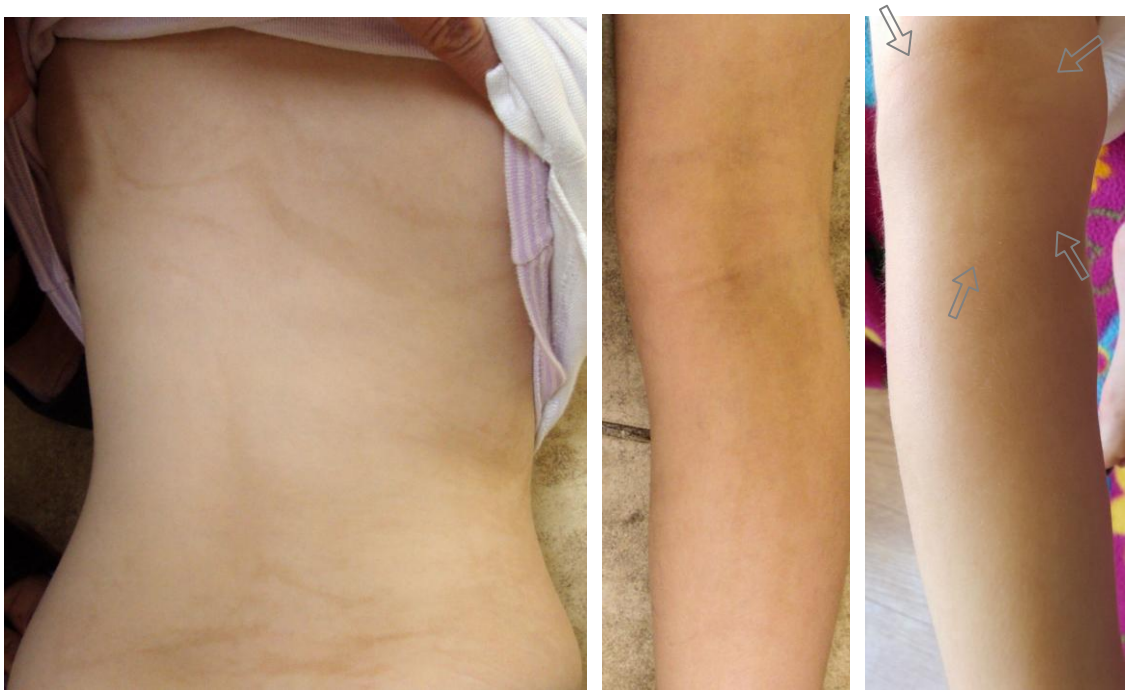
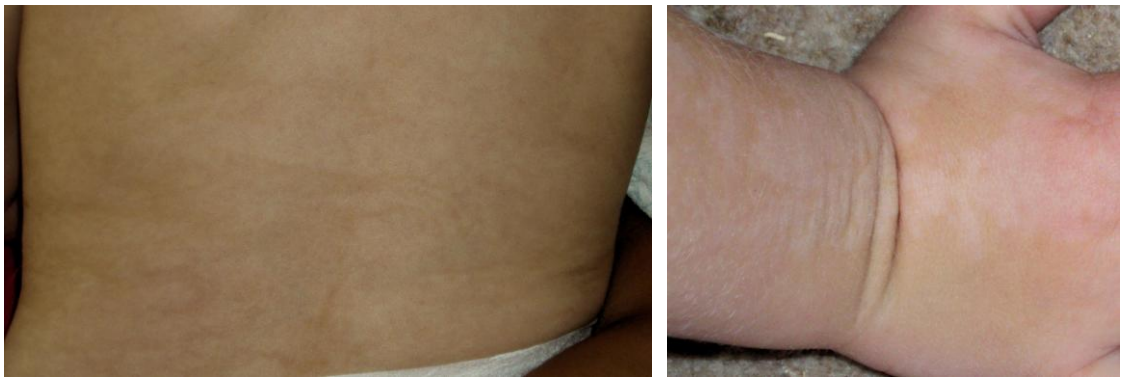


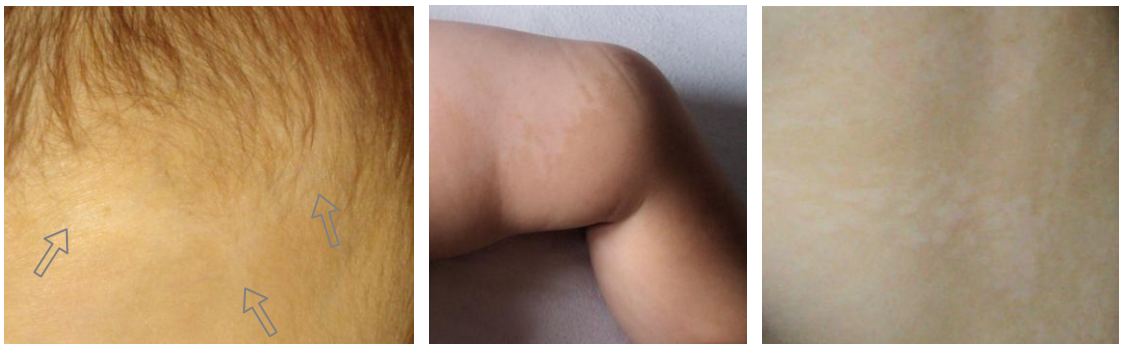
Figure 32: Examples of skin pigmentary abnormalities



Note the hyperpigmentation that follows Blashko's lines in the first two photographs. The third shows subtle, blotchy hypopigmentation within the arrowed region.



Both of these photographs show patchy hyperpigmentation.



The first photograph shows a subtle, V-shaped area of hypo pigmentation, indicated by the arrows. The other photographs show patchy hyperpigmentation.

4k: Summary of Phenotype

Table 14 below summarises the features seen in the study patients in comparison to those recorded in the literature reports of patients with mosaic tetrasomy and/ or hexasomy for an isochromosome 12p. It does not include those with more atypical karyotypical abnormalities.

Table 14: Comparison of phenotype in reported cases with mosaic tetrasomy/ hexasomy for i(12p) and in study group

	Reported as neonates (n=31)		Reported when older (n=76)		Study group (n=21 or 22*)	
	Present**	Absent**	Present**	Absent**	Present**	Absent**
General and neurological						
Polyhydramnios	21 (67.7%)	0	13 (17.1%)	0	4 (18.2%)	12 (54.5%)
Birth weight: high	6 (19.4%)	19 (61.3%)	10 (13.2%)	51 (67.1%)	3 (13.6%)	19 (86.4%)
Birth weight: IUGR	3 (9.7%)	22 (71.0%)	0	61 (80.3%)	0	22 (100%)
Macrocephaly at birth	5 (16.1%)	10 (32.3%)	1 (1.3%)	25 (32.9%)	3 (13.6%)	11 (50.0%)
Microcephaly at birth	1 (3.2%)	14 (45.2%)	0	28 (36.8%)	0	14 (63.6%)
Birth length: high	0	14 (45.2%)	6 (7.9%)	36 (47.4%)	1 (4.5%)	6 (27.3%)
Birth length: low	1 (3.2%)	13 (41.9%)	1 (1.3%)	41 (53.9%)	0	7 (31.8%)
Feeding difficulties	2 (6.5%)	0	12 (15.8%)	2 (2.6%)	15 (68.2%)	7 (31.8%)
Failure to thrive	0	0	8 (10.5%)	6 (7.9%)	4 (18.2%)	18 (81.8%)
Seizures	1 (3.2%)	0	39 (51.3%)	8 (10.5%)	16 (72.7%)	6 (27.3%)
Macrocephaly	n/a	n/a	7 (9.2%)	47 (61.8%)	2 (9.5%)	19 (90.5%)
Microcephaly	n/a	n/a	9 (11.8%)	45 (59.2%)	6 (28.6%)	15 (71.4%)
Ventriculomegaly/ atrophy	8 (25.8%)	4 (12.9%)	30 (39.5%)	12 (15.8%)	3 (13.6%)	11 (50.0%)
Other intracranial abnormalities	7 (22.6%)	3 (9.7%)	10 (13.2%)	17 (22.4%)	4 (18.2%)	10 (45.5%)

	Reported as neonates (n=31)		Reported when older (n=76)		Study group (n=21 or 22*)	
	Present**	Absent**	Present**	Absent**	Present**	Absent**
Hypotonia as infant	3 (9.7%)	0	51 (67.1%)	1 (1.3%)	20 (90.9%)	2 (9.1%)
Later hypertonia	n/a	n/a	6 (7.9%)	0	1 (4.8%)	20 (95.2%)
Mental retardation	n/a	n/a	67 (88.2%)	0	22 (100%)	0
-profound	n/a	n/a	11 (14.5%)	0	7 (31.8%)	15 (68.2%)
-severe	n/a	n/a	13 (17.1%)	0	3 (13.6%)	
-mild	n/a	n/a	4 (5.3%)	0	6 (27.3%)	
Absent speech	n/a	n/a	17 (22.4%)	5 (6.6%)	6 (27.3%)	16 (72.7%)
Hearing impairment	0	0	19 (25.0%)	4 (5.3%)	17 (77.3%)	4 (18.2%)
Visual impairment	0	0	6 (7.9%)	0	17 (77.3%)	5 (22.7%)
Nystagmus	0	0	7 (9.2%)	1 (1.3%)	5 (22.7%)	17 (77.3%)
Optic nerve atrophy/ hypoplasia	0	0	3 (3.9%)	4 (5.3%)	3 (13.6%)	0
Retinal pallor	0	0	3 (3.9%)	3 (3.9%)	1 (4.5%)	0
Hypopigmented lesions	1 (3.2%)	1 (3.2%)	42 (55.3%)	8 (10.5%)	13 (61.9%)	8 (38.1%)
Hyperpigmented lesions	0	1 (3.2%)	22 (28.9%)	7 (9.2%)	11 (52.4%)	10 (47.6%)
Lymphoedema	3 (9.7%)	0	8 (10.5%)	1 (1.3%)	1 (4.8%)	20 (95.2%)
Redundant/ lax skin	13 (41.9%)	3 (9.7%)	14 (18.4%)	2 (2.6%)	0	21 (100%)
Areas of hypertrichosis	1 (3.2%)	0	4 (5.3%)	0	0	21 (100%)
Facial features						
Prominent forehead	9 (29.0%)	0	60 (78.9%)	0	0	21 (100%)
Sparse hair/ areas of alopecia	11 (35.5%)	1 (3.2%)	61 (80.3%)	1 (1.3%)	15 (71.4%)	6 (28.6%)
Sparse eyebrows	3 (9.7%)	0	33 (43.4%)	3 (3.9%)	4 (19.0%)	17 (81.0%)
Synophrys	0	3 (9.7%)	4 (5.3%)	33 (43.4%)	0	21 (100%)
Sparse eyelashes	1 (3.2%)	2 (6.5%)	7 (9.2%)	3 (3.9%)	3 (14.3%)	18 (85.7%)

	Reported as neonates (n=31)		Reported when older (n=76)		Study group (n=21 or 22*)	
	Present**	Absent**	Present**	Absent**	Present**	Absent**
Hypertelorism/ telecanthus	20 (64.5%)	1 (3.2%)	54 (71.1%)	2 (2.6%)	12 (57.1%)	9 (42.9%)
Up-slanting palpebral fissures	3 (9.7%)	2 (6.5%)	20 (26.3%)	13 (17.1%)	9 (42.9%)	12 (57.1%)
Short palpebral fissures	5 (16.1%)	0	9 (11.8%)	0	4 (19.0%)	17 (81.0%)
Epicanthic folds	5 (16.1%)	0	32 (42.1%)	5 (6.6%)	4 (19.0%)	17 (81.0%)
Ptosis	0	0	10 (13.2%)	1 (1.3%)	5 (23.8%)	16 (76.2%)
Cataracts	0	3 (9.7%)	3 (3.9%)	6 (7.9%)	0	21 (100%)
Corneal clouding	3 (9.7%)	0	1 (1.3%)	8 (10.5%)	0	21 (100%)
Flat nasal bridge	11 (35.5%)	0	34 (44.7%)	1 (1.3%)	13 (61.9%)	8 (38.1%)
Broad nasal bridge	9 (29.0%)	0	32 (42.1%)	2 (2.6%)	1 (4.8%)	20 (95.2%)
Short nose	11 (35.5%)	0	24 (31.6%)	2 (2.6%)	20 (95.2%)	1 (4.8%)
Anteverted nares	11 (35.5%)	0	40 (52.6%)	2 (2.6%)	10 (47.6%)	11 (52.4%)
Full cheeks	1 (3.2%)	0	20 (26.3%)	1 (1.3%)	5 (23.8%)	16 (76.2%)
Long philtrum	9 (29.0%)	1 (3.2%)	33 (43.4%)	5 (6.6%)	15 (71.4%)	6 (28.6%)
Macrostomia	4 (12.9%)	0	24 (31.6%)	1 (1.3%)	3 (14.3%)	18 (85.7%)
Cupid’s bow appearance to mouth	3 (9.7%)	0	15 (19.7%)	0	12 (57.1%)	9 (42.9%)
Cleft lip	1 (3.2%)	13 (41.9%)	0	25 (32.9%)	0	21 (100%)
Cleft palate	5 (16.1%)	9 (29.0%)	6 (7.9%)	20 (26.3%)	2 (9.5%)	19 (90.5%)
Cleft uvula	1 (3.2%)	0	5 (6.6%)	1 (1.3%)	1 (4.8%)	20 (95.2%)
High arched palate	10 (32.3%)	0	20 (26.3%)	3 (3.9%)	7 (33.3%)	14 (66.7%)
Macroglossia	2 (6.5%)	0	22 (28.9%)	3 (3.9%)	5 (23.8%)	16 (76.2%)
Micrognathia	6 (19.4%)	0	9 (11.8%)	15 (19.7%)	4 (19.0%)	17 (81.0%)
Prognathia	0	6 (19.4%)	13 (17.1%)	11 (14.5%)	6 (28.6%)	15 (71.4%)
Low-set ears	18 (58.1%)	0	28 (36.8%)	5 (6.6%)	12 (57.1%)	9 (42.9%)
Posteriorly rotated ears	7 (22.6%)	0	13 (17.1%)	5 (6.6%)	8 (38.1%)	13 (61.9%)

	Reported as neonates (n=31)		Reported when older (n=76)		Study group (n=21 or 22*)	
	Present**	Absent**	Present**	Absent**	Present**	Absent**
Small ears	10 (32.3%)	0	11 (14.5%)	7 (9.2%)	3 (14.3%)	18 (85.7%)
Large ears	0	10 (32.3%)	4 (5.3%)	15 (19.7%)	0	21 (100%)
Coarse face	9 (29.0%)	0	30 (39.5%)	2 (2.6%)	11 (52.4%)	10 (47.6%)
Skeleton and limbs						
Short stature	n/a	n/a	20 (26.3%)	22 (28.9%)	5 (22.7%)	17 (77.3%)
Craniosynostosis	0	0	3 (3.9%)	2 (2.6%)	0	22 (100%)
Large fontanelles/ delayed closure	2 (6.5%)	0	12 (15.8%)	2 (2.6%)	12 (54.5%)	7 (33.3%)
Delayed eruption of teeth	n/a	n/a	4 (5.3%)	1 (1.3%)	12 (54.5%)	10 (45.5%)
Short neck	13 (41.9%)	0	16 (21.1%)	0	2 (9.5%)	19 (90.5%)
Scoliosis/ kyphosis	0	0	10 (13.2%)	1 (1.3%)	6 (27.3%)	16 (72.7%)
<12 pairs of ribs	6 (19.4%)	0	1 (1.3%)	0	2 (9.5%)	19 (90.5%)
Short limbs	9 (29.0%)	2 (6.5%)	7 (9.2%)	3 (3.9%)	5 (23.8%)	16 (76.2%)
-Rhizomelic	3 (9.7%)	0	4 (5.3%)	0	3 (14.3%)	18 (85.7%)
Short hands and feet	8 (25.8%)	3 (9.7%)	25 (32.9%)	1 (1.3%)	12 (57.1%)	9 (42.9%)
Polydactyly of fingers	1 (3.2%)	0	5 (6.6%)	0	0	21 (100%)
Clinodactyly	3 (9.7%)	0	7 (9.2%)	0	4 (19.0%)	17 (81.0%)
Single palmar creases	4 (12.9%)	0	12 (15.8%)	2 (2.6%)	10 (47.6%)	11 (52.4%)
-Unilateral	4 (12.9%)		6 (7.9%)		4 (19.0%)	
-Bilateral	0		6 (7.9%)		6 (28.6%)	
Delayed bone age	1 (3.2%)	0	5 (6.6%)	1 (1.3%)	1 (4.5%)	
Hypoplastic nails	7 (22.6%)	2 (6.5%)	8 (10.5%)	1 (1.3%)	1 (4.8%)	20 (95.2%)
Polydactyly of toes	3 (9.7%)	0	4 (5.3%)	0	0	22 (100%)
Hip dislocation	0	0	11 (14.5%)	0	3 (13.6%)	19 (86.4%)
Contractures	5 (16.1%)	0	11 (14.5%)	2 (2.6%)	3 (13.6%)	19 (86.4%)

	Reported as neonates (n=31)		Reported when older (n=76)		Study group (n=21 or 22*)	
	Present**	Absent**	Present**	Absent**	Present**	Absent**
Features in thorax and abdomen						
Cardiac defects	8 (25.8%)	4 (12.9%)	20 (26.3%)	12 (15.8%)	4 (18.2%)	16 (72.7%)
Accessory nipples	2 (6.5%)	2 (6.5%)	14 (18.4%)	12 (15.8%)	9 (42.9%)	12 (57.1%)
Diaphragmatic hernia	19 (61.3%)	1 (3.2%)	2 (2.6%)	0	0	22 (100%)
Accessory spleens	5 (16.1%)	3 (9.7%)	0	5 (6.6%)	0	0
Exomphalos	4 (12.9%)	1 (3.2%)	2 (2.6%)	25 (32.9%)	0	22 (100%)
Umbilical hernia	0	0	18 (23.7%)	8 (10.5%)	5 (22.7%)	17 (77.3%)
Inguinal hernia	0	0	9 (11.8%)	1 (1.3%)	2 (9.1%)	20 (90.9%)
Imperforate anus	8 (25.8%)	3 (9.7%)	4 (5.3%)	3 (3.9%)	0	21 (100%)
Hypospadias***	1 (6.3%)	3 (18.8%)	0	4 (7.0%)	0	14 (100%)
Undescended testes***	7 (43.8%)	2 (12.5%)	13 (22.8%)	4 (7.0%)	4 (28.6%)	10 (71.4%)

*Due to the inclusion of the patient whose family chose to participate after his death, the numerator is 22 for items covered in the history but 21 for items covered in the examination.

**The figures in the present and absent columns represent the cases where the literature report makes it clear that the feature was present or absent respectively. These figures rarely add up to 100% as few of the case reports contain all of the information sought for this literature summary. Equally, there are some items where absence of a feature could not be confirmed in the study group.

***As these features are only seen in males, the percentages for these entries are calculated for the males in each group.

4l: Diagnosis And Cytogenetics Results

Diagnosis

Most of the patients were diagnosed in infancy. As previously mentioned, one of the babies had been diagnosed antenatally. Eight (36.4%) were diagnosed in the first year of life, six (27.3%) in the second and three (13.6%) in the third. The other three children were diagnosed at four, five and 15 years of age. The diagnosis in the 15 year old was suspected at a much younger age but her parents chose not to proceed with investigations at that stage.

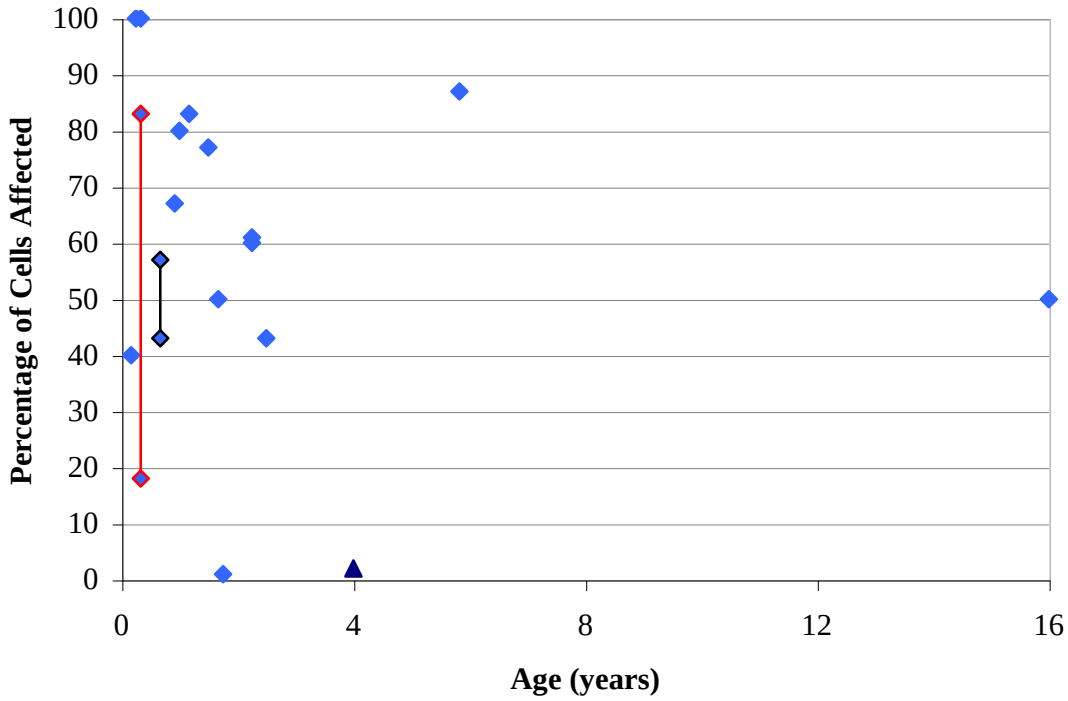
Those with a mild phenotype were all diagnosed before their sixth birthdays, with the youngest being diagnosed antenatally. Excluding this patient, the mean age of diagnosis in this group was just before the third birthday. In the moderate phenotype group, the age of diagnosis varied from four months to 15 years, with a mean of two years and nine moths if the oldest child is included and 15 months if she is excluded from the analysis. For the severe phenotype group, the age of diagnosis ranged from two months to two years, with a mean of nine months.

In 13 cases (59.1%), the parents were aware that the diagnosis was suspected and that confirmatory testing was being undertaken. The diagnosis was made after a skin biopsy in 18 cases (81.8%). Two of these (9.1% of total group) had already had array CGH, which had suggested an increased dosage of 12p and the skin biopsy was performed for confirmation. One child had been diagnosed antenatally on an amniocentesis sample and three (13.6%) were diagnosed after FISH testing of the buccal mucosa.

Although 18 patients had previously had a skin biopsy, it was only possible to obtain the results from 17 of these. In the other case, neither of the two possible cytogenetics laboratories could trace the sample. Two of the older results, from skin biopsies taken approximately 20 years ago, simply stated that ‘about 50%’ of cells were tetrasomic. One of the patients had no tetrasomic cells detected but three out of 140 cells were hexasomic for 12p. Two of the skin biopsy sample results documented that two separate cultures had been analysed and provided the results of these separately. These showed seven and 33 out of 40 cells affected in one patient (17.5% and 82.5%) and 24

and 32 out of 56 cells affected in the other patient (42.9% and 57.1%). The results of the skin biopsies are shown in Figure 33 below.

Figure 33: Percentage of affected cells in skin biopsy



Key:

The diamonds represent tetrasomic cells and the triangles hexasomic cells. Those joined with vertical lines show two separate analyses from the same skin biopsy.

Array CGH

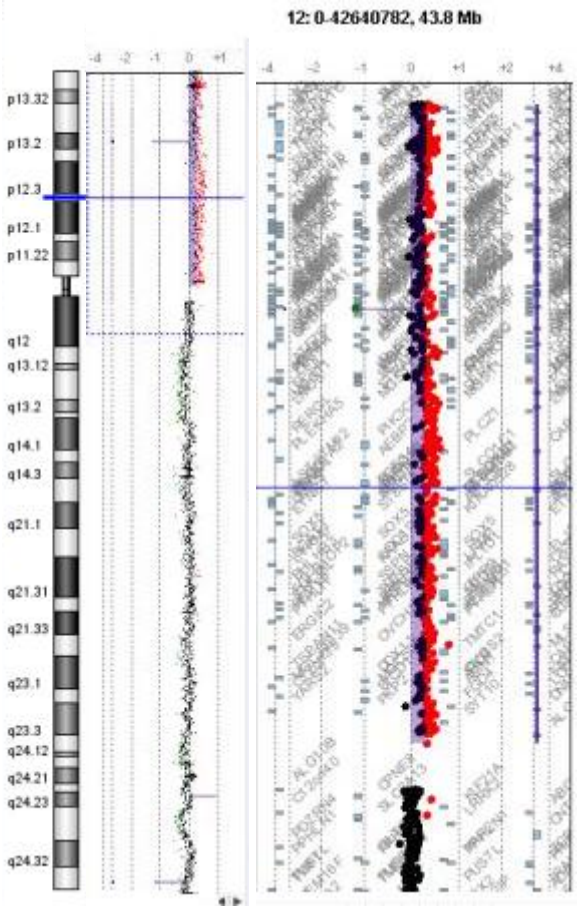
Of the 21 living patients with Pallister-Killian syndrome recruited, 16 (76.2%) agreed to a blood sample being obtained from the patient. One patient had already had array CGH testing in our laboratory so a repeat sample was not requested and his original array CGH results are included below. In another two cases, a stored sample was obtained from a different laboratory with parental consent. Array CGH testing was therefore carried out on 19 (90.5%) of the patients. The fetal sample was not included since the laboratory did not have experience in performing array CGH testing on fetal samples.

One of the stored samples obtained from a different laboratory gave a poor quality profile that could not be interpreted despite passing the initial checks of DNA quality. This is a known risk of using old samples and those obtained from other laboratories, as differences in the DNA extraction methods affect the quality of the array profile.

Array CGH results were obtained on 18 (85.7%) of the living patients. Only three of them, including the two who had initially been diagnosed after array CGH, showed any evidence of the presence of additional 12p material. These had $\log_2$ ratios of 0.117352, 0.291972 and 0.6 suggesting approximately 8.5%, 22.5% and 50% of cells had tetrasomy for 12p (equivalent to 17%, 45% and 100% having trisomy 12p). See Figure 34 for an example of this.

Where the array CGH detected the presence of additional 12p material, it showed an increase in dosage along the full length of 12p.

Figure 34: Example of mosaic increase in copies of 12p



Key:

The ideogram on the left shows the full length of chromosome 12, with the blue line representing the centre of the region enlarged on the right. The dotted blue box in the middle figure denotes the full region enlarged on the right (the top line of the box is obscured by the solid black line at the top of the diagram but the vertical dotted, blue line is clearly visible.) The black dots on the right hand diagram show normal dosage of 12q (approximating 0 on the $\log_2$ scale), while the red dots represent the increased dosage of 12p. The $\log_2$ ratio in this case is 0.291972 suggesting either that approximately 45% of cells had trisomy 12p or approximately 22.5% of cells had tetrasomy 12p.

Normal results were obtained in 11 cases (61.1%). The other four had no evidence of additional 12p material but had other array abnormalities. A second array abnormality was also detected in one of those with evidence of additional 12p material. This means that five of the 18 patients (27.8%) tested had array abnormalities unrelated to 12p and

this is in keeping with the estimated 25% abnormality rate found on array CGH testing[134;135]. One of these abnormalities was a terminal deletion of 5p of between 116 and 197kb, an area with no known genes, and is believed to be a polymorphism. One child had a de novo duplication of approximately 3.16Mb involving 16p13.11 to p12.3, which is likely to have some clinical significance due to its size, the number of genes involved and reports of a smaller (1.5Mb) duplication of 16p13.1 being associated with mental retardation and autism[136]. Duplication of 16p13.1 has been associated with an increased risk of schizophrenia[137]. A third had a deletion of 17p12 of between 1.3 and 1.5Mb, which encompasses the critical region for hereditary neuropathy with liability to pressure palsies, which should be clinically significant. The other two cases were found to have copy number variants of unknown significance. One had a duplication of between 325 and 446kb involving 14q24.3, while the second had three duplications involving the sex chromosomes. These involved Xq22.3, Xq23 and the part of the pseudoautosomal region of either Xp22.33 or Yp11.32 and ranged in size from a minimum of 447kb to a maximum of 676kb. The genes involved in each of these deletions or duplications are shown in Table 15 below.

Table 15: Other detected array abnormalities and genes involved

5p15.33 deletion (116- 197kb)

No known genes

14q24.3 duplication (325- 446kb)

VASH1 (not known to cause disease)

ANGEL1 (not known to cause disease)

Part of *ESRRB* (autosomal recessive deafness)

16p13.11p12.3 duplication (approximately 3.16Mb)- de novo

PDXDC1 (not known to cause disease)

NTAN1 (not known to cause disease)

RRN3 (not known to cause disease)

MPV17L (autosomal recessive hepatorenal mitochondrial DNA depletion syndrome[138])

C16orf45 (not known to cause disease)

KIAA0430 (not known to cause disease)

NDE1 (point mutations cause autosomal recessive microlissencephaly[139])

MYH11 (point mutations cause aortic aneurysms/ dissections, along with patent ductus arteriosus[140])

C16orf63 (not known to cause disease)

ABCC1 (not known to cause disease)

ABCC6 (point mutations cause pseudoxanthoma elasticum[141])

NOMO1 (not known to cause disease)

XYLT1 (variants affect the severity of the phenotype in pseudoxanthoma elasticum[142])

17p12 deletion (1.3- 1.5Mb)

COX10 (point mutations cause autosomal recessive Leigh syndrome[143])

CDRT15 (not known to cause disease)

HS3ST3B1 (not known to cause disease)

PMP22 (deletion, as in this patient, causes Hereditary neuropathy with liability to pressure palsies; duplication causes Charcot-Marie-tooth disease)

TEKT3 (not known to cause disease)

CDRT4 (not known to cause disease)

FAM18B2 (not known to cause disease)

Xq22.3 duplication (479- 592kb)

CXorf41 (not known to cause disease)

NUP62CL (not known to cause disease)

MYCL2 (not known to cause disease)

FRMPD3 (not known to cause disease)

PRPS1 (loss of function mutations cause Charcot-Marie-tooth disease, Arts syndrome & deafness; gain of function mutations cause gout & phosphoribosylpyrophosphate synthetase superactivity[144])

Xq23 duplication (447- 656kb)

Part of *TRPC5* (not known to cause disease)

Xp22.33 or Yp11.32 (pseudoautosomal region) duplication (659- 676kb)

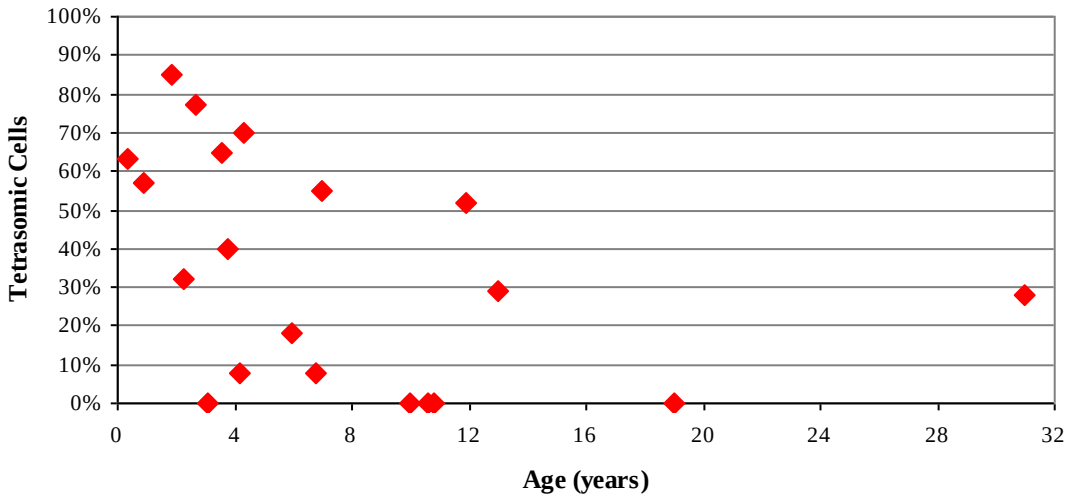
Part of *PPP2R3B* (not known to cause disease)

SHOX (deletion causes Leri-Weill dsychondrosteosis but duplication, as in this patient, not thought to cause disease)

FISH Analysis

It was possible to obtain buccal mucosa samples from 20 of the patients seen as part of this study. These confirmed the diagnosis in 15 cases (75%), while the other five (25%) had only two chromosome 12 centromere signals in each of the 100 cells examined. Of the three who had originally been diagnosed on buccal mucosa FISH, two (66.6%) had positive results in the study analysis. One patient who had previously had a skin biopsy showing that 24 out of 30 cells (80%) were tetrasomic was found to have tetrasomy in 25 cells (out of 67; 37%) and hexasomy in two cells (3%). The percentage of tetrasomic or hexasomic cells found is shown in comparison to age in Figure 35. Table 18 (page 138) shows the correlation between these results and the results of the other cytogenetics tests.

Figure 35: Tetrasomic or hexasomic cells seen on buccal FISH and patient age



Many of the patients were incontinent and it was difficult to obtain a urine sample from some of those who were continent. The laboratory staff also found the urine samples difficult to process so this was abandoned after the first three samples had been processed. From the three samples submitted, it was only possible to identify 100 cells for analysis in one individual. That patient had tetrasomy 12p in 52 of the cells identified in the urine (52%), which was very similar to the 55% identified on the buccal FISH analysis. In the other two individuals whose urine samples were processed, only two and 27 cells were identified and no tetrasomic cells were detected.

These patients had shown 18% and 0% tetrasomic cells respectively on the buccal mucosal analysis.

Fetal Results

Samples were also available from the terminated fetus' skin, umbilical cord, amnion and chorionic villi. The results of these are shown in Table 16 along with the previous amniocentesis results.

Table 16: Tetrasomic cells in fetus

Tissue	Tetrasomic Cells	Normal Cells	% Abnormal Cells
Amniotic fluid	3	23	12%
Amnion	20	32	38%
Chorionic villi	19	111	15%
Umbilical cord	0	136	0%
Fetal skin	20	30	40%

One of the cytogenetics laboratories also supplied a detailed breakdown of the results found in their two terminated fetuses and these are shown in below. The one labelled as 'other fetus 2' was noted to have had a small number of cells for analysis in all except the blood sample.

Table 17: Percentage tetrasomic cells in three fetuses

Tissue	Fetus in Study	Other Fetus (1)	Other Fetus (2)
Amniotic fluid	12%		
Amnion	38%	25%	100%
Chorionic villi	15%		
Umbilical cord	0%		100%
Fetal skin/ fascia	40%	15%	100%
Pericardium		70%	100%
Ovary		100%	100%
Cardiac blood		11%	4%

Table 18 shows details of the old skin biopsy results, along with the buccal mucosa FISH and array CGH results from this study.

Table 18: Cytogenetic results in order of buccal mucosa results

Buccal mucosa	Urine	Array	Previous Skin Biopsy
85%			100%
77%		Increased 12p dosage (estimated 8.5%)	61%
70%		Normal	77%
65%		Increased 12p dosage (estimated 50%); Other variant found	60%
63%			
57%		Increased 12p dosage (estimated 22.5%)	18% & 83%
55%	52%	Normal	87%
52%		Normal	100%
40%*		Normal	80%
32%		Normal	
29%		Normal	67%
28%		Other variant found	about 50%
18%	0%	Other variant found	2%
8%		Other variant found	
8%		Normal	40%
0%		Normal	
0%		Normal	
0%	0%	Normal	97%
0%		Other variant found	43%
0%		Normal	83%
		Normal	43% & 57%
			about 50%

Blank cells represent those where the testing was not performed or where no result is available and 0% those where no tetrasomic cells were identified.

*37% tetrasomic cells plus 3% hexasomic cells

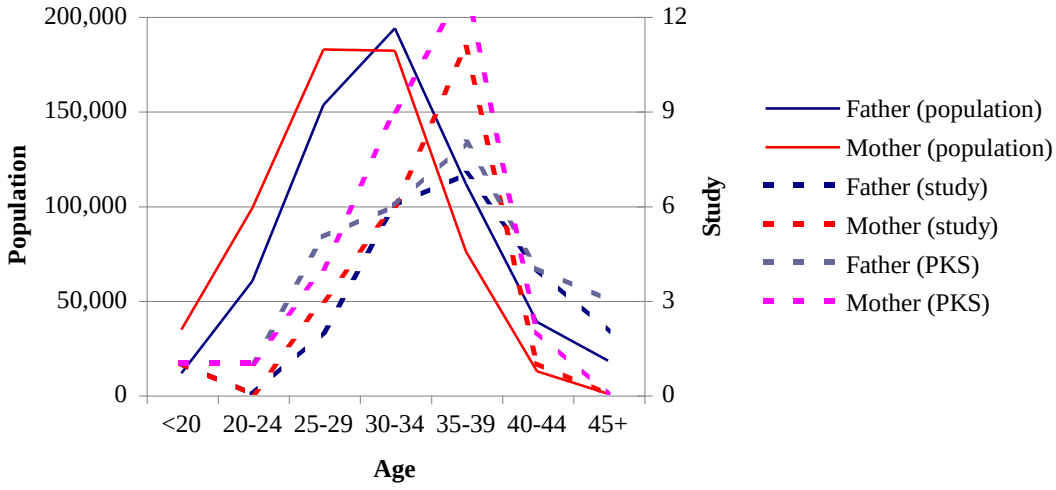
4m: Parental Ages And Molecular Genetics Results

Parental Ages

Parental ages at the birth of the patient with Pallister-Killian syndrome were obtained from the parent(s) present at the study visit. This data was also available for the family who chose to participate in the study after undergoing a termination of pregnancy for Pallister-Killian syndrome. The laboratories were also able to supply this data, or the information to calculate it, in a small number of cases, mainly where a termination of pregnancy had occurred. Overall, the data supplied by the laboratories added the age at delivery of an additional seven mothers and five of the fathers.

This data is shown graphically below, with the 'PKS' group representing both those who participated in the study and those whose ages at delivery/ termination of pregnancy were obtained from the laboratories. The 'study' group only includes the live born individuals who participated in the study, as the population data is for live births.

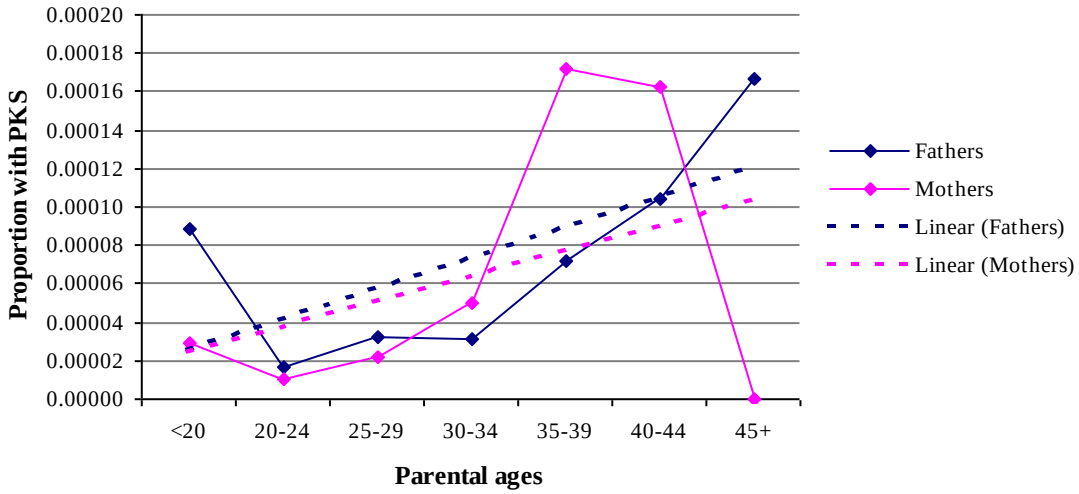
Figure 36: Parental ages at delivery in comparison to population



Note that the maternal and paternal age curves for babies with Pallister-Killian syndrome are shifted to the right of the population curves, whether using the data from the study participants or all available data.

Figure 37 shows the number of babies born with Pallister-Killian syndrome as a proportion of the live births in each maternal/ paternal age group, along with the linear regression line for these proportions.

Figure 37: Proportion of babies with Pallister-Killian syndrome by parental ages



The binary linear regression analyses are shown in Figure 44 to Figure 51 in Appendix 2 (page 203). In each analysis the initial analysis using the five-year age bands shows that not all age bands include affected births, which increases the risks of spurious results. This has been corrected in each case by amalgamating the age bands to produce wider age ranges that all include affected births. In the case of the mothers, only three age bands (less than 30, 30 to 34 and at least 35 years) could be used to maintain the number of affected births in each age band at a minimum of two. For the fathers, it was possible to achieve this while maintaining four age bands (less than 30, 30 to 34, 35 to 39 and at least 40 years).

All analyses using the reduced number of age bands for both maternal and paternal age showed a statistically significant increase in the risk of a baby having Pallister-Killian syndrome with increasing parental age. The p values for the maternal analyses were less than 0.001 for both the study group and when encompassing all available data, while the p values for the paternal analyses were 0.016 and 0.024 respectively. Table 19 and Table 20 summarise the calculated odds ratios in comparison to the risks in parents aged less than 30 years. The full SPSS data is shown in Appendix 2 (page 203).

Table 19: Odds ratios for Pallister-Killian syndrome by increasing maternal age

Age band	Study group	All data
<30 years	1.0	1.0
30- 34 years*	2.6 (0.7- 9.2)	2.6 (0.9- 7.3)
35+ years*	10.7 (3.5- 33.1)	8.9 (3.4- 23.0)

*These risks are in comparison to the risk for those aged less than 30 years.

Table 20: Odds ratios for Pallister-Killian syndrome by increasing paternal age

Age band	Study group	All data
<30 years	1.0	1.0
30- 34 years*	2.3 (0.6- 9.3)	1.0 (0.3- 3.0)
35- 39 years*	4.7 (1.2- 18.2)	2.3 (0.8- 6.3)
40+ years*	8.0 (2.0- 31.8)	4.0 (1.4- 11.3)

*These risks are in comparison to the risk for those aged less than 30 years.

Microsatellite Results

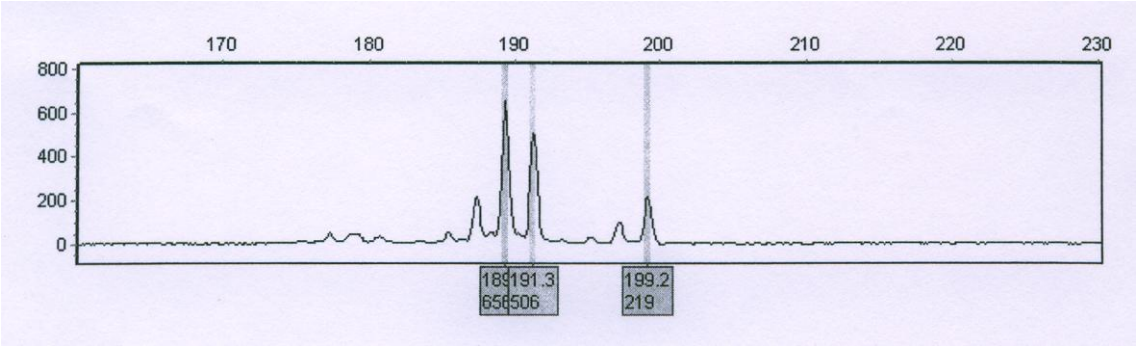
DNA samples were available for 18 patients with Pallister-Killian syndrome at the time of this analysis. Where it was clear at the time of the visit that it would not be possible to obtain DNA from the patient or from one of the parents, no parental samples were taken. In two cases, DNA samples were already stored in the laboratory and blood samples were obtained from both parents at the study visit in five cases. In another nine cases, blood was taken from the mother during the visit and a blood form left for the father, five (62.5%) of whom had samples taken at a later date.

The results of the microsatellite analysis are shown in Table 28 (page 219), with those where both parental samples were available shown first, followed by those where a maternal sample was obtained and then by those where no parental samples were obtained. Each of these three groups is then ordered by age. All patients analysed were heterozygous for at least one of the three 12q microsatellite markers analysed.

These show that two patients (11.11%) had evidence of having inherited three separate alleles at one (E) or two (R) microsatellite loci. An example of this is shown in Figure 38. The patient with evidence of having inherited three separate alleles at two microsatellite loci also had an increased dosage of one allele at a third locus but parental

samples were unavailable. In the other case it is unclear from which parent the additional allele was inherited as the patient had one allele that was present only in the mother, one that was present only in the father and one that was present in both parents.

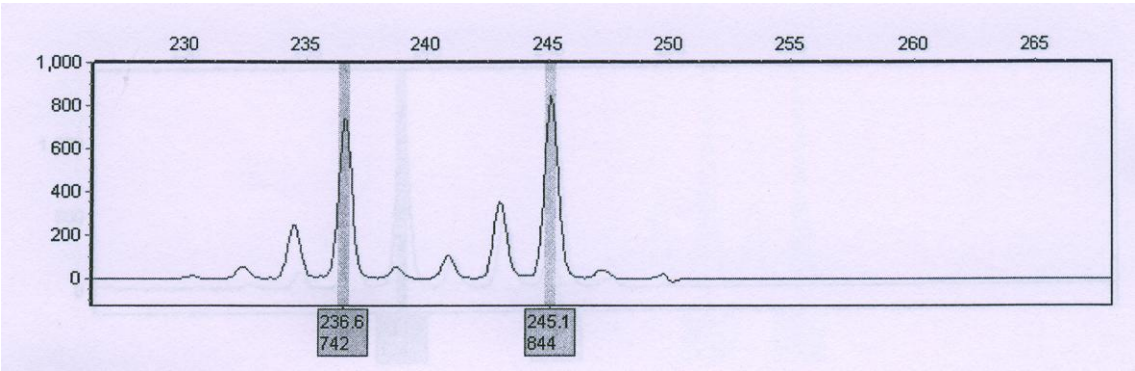
Figure 38: Microsatellite analysis showing presence of three alleles



The grey vertical lines indicate the three peaks that have been identified by the GeneMarker 1.85 software.

Seven patients (38.9%), including the one mentioned above, showed evidence of an increased dosage of one allele at a minimum of one microsatellite marker, as displayed in Figure 39. This applied to a single microsatellite marker in three cases, two markers in two cases, three markers in one case and four markers in the final case. It is not clear whether the abnormalities were not detected at the other loci or whether the results with only one or two markers showing an increased dosage of one allele represent artefacts.

Figure 39: Microsatellite analysis showing increased dosage of the larger allele



The grey vertical lines indicate peaks that have been identified by the GeneMarker 1.85 software. Note that the larger (245.1) allele has produced a higher peak than the 236.6, suggesting that it is present in a higher dosage, as preferential amplification of the smaller allele is expected. (Refer back to Figure 8 for a normal control sample.)

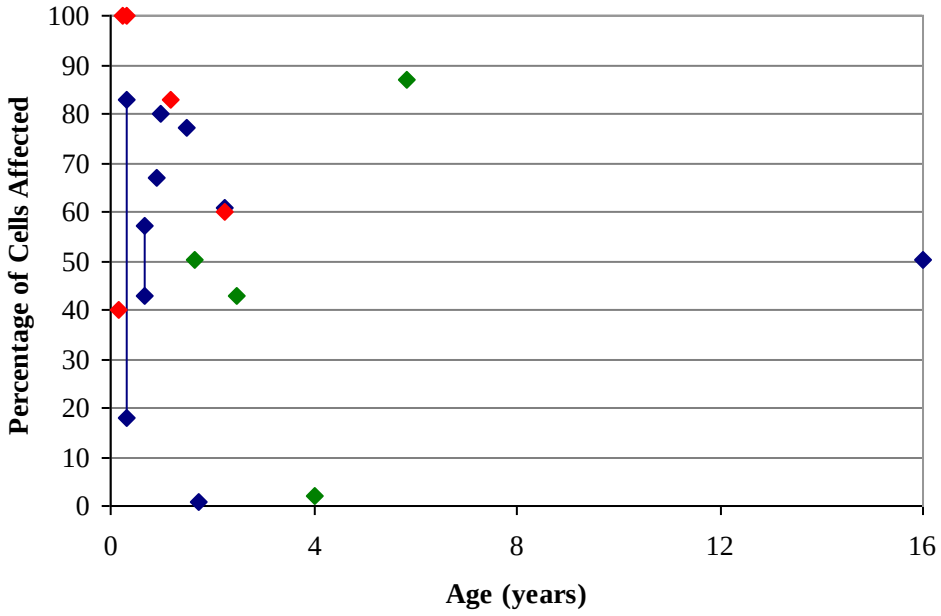
As Table 28 (page 219) shows, in the case (M) with four markers showing an apparent increased dosage, three of these were maternal and one was paternal. In the case of the patient with three markers (J) showing an apparent increased dosage, all three were of paternal origin. Paternal origin was also suggested by one of the two cases with an increased dosage of two markers (F), while the other (Q) had no parental samples available. The cases with only one marker suggestive of an increased dosage would imply maternal origin (H) or are uninformative due to a shared parental allele (K) or, in the case with evidence of having inherited three separate alleles at two other microsatellite loci (R), the absence of parental samples.

4n: Genotype-Phenotype Correlation

As mentioned in 4l: Diagnosis And Cytogenetics Results, two patients' skin biopsy samples had been divided and analysed in two separate cultures. Each of these showed discrepant results (17.5% compared to 82.5% in one patient and 42.9% compared to 57.1% in the other).

The skin biopsy results showed that the proportion of tetrasomic cells in the mild phenotype group was 2- 87% (mean 45.5%). The moderate phenotype group includes the two patients mentioned above. Including each of their cell cultures separately, the range for this group is 17.5- 97% (mean 63.2%), while taking the mean of the two cultures for each patient gives a range of 50- 97% (mean 66.5%). For the severe phenotype group, the proportion of tetrasomic cells was 40- 100% (mean 76.6%).

Figure 40: Skin biopsy results by phenotype



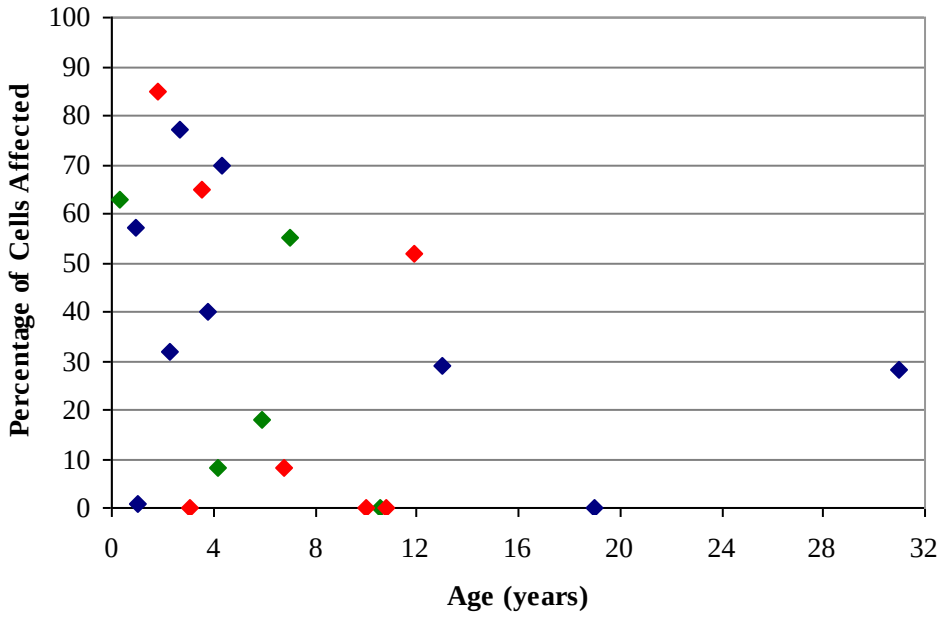
Key:

Red- severe phenotype; blue- moderate phenotype (the vertical bars link the discordant results on one sample); green- mild phenotype

Likewise, the buccal mucosa FISH analysis showed ranges of 0- 63% (mean 28.8%) abnormal cells in the mild phenotype group, 0- 77% (mean 41.6%) in the moderate

phenotype group and 0- 85% (mean 30.0%) in the severe phenotype group. One of the patients in the moderate group had 37% trisomic cells and 3% hexasomic cells.

Figure 41: Buccal FISH results by phenotype



Key:
Red- severe phenotype; blue- moderate phenotype; green- mild phenotype

Of those with a detectable increase in 12p dosage on array CGH testing, one was in the severe group (four analysed; 25.0%) and two were in the moderate group (nine analysed; 22.2%). None of the five analysed in the mild phenotype group would have been diagnosed by array CGH.

None of the patients were shown to have uniparental disomy for 12q so it is not possible to assess any potential effect of this. Microsatellite analysis confirmed the presence of three alleles in one of the patients in the severe phenotype group (of the six analysed; 16.7%).

4o: Child With Trisomy 12p

One six year-old child recruited to the study had an unbalanced translocation. This resulted in trisomy of the terminal portion of 12p (approximately 18Mb) and monosomy for the 1q telomere, rather than Pallister-Killian syndrome. His karyotype was 46XY,der(1)t(1;12)(q44;p12.3) mat.

There was no polyhydramnios, nor any antenatally detected congenital anomalies. He was born at term with a birth weight on the 50th centile. Bilateral inguinal herniae developed and intra-hepaptic bile duct hypoplasia was detected. There were no other congenital anomalies but his dentition was delayed. An echocardiogram and a renal ultrasound scan had both been normal.

All of his growth measurements showed a relatively large build, with heights and weights ranging from the 75th to the 99.6th centiles throughout his life. His head circumference at the time of the study meeting was on the 9th centile. He had global developmental delay and some autistic traits. He smiled at seven weeks of age and began walking with his hands held at 21 months of age. At six years old, his developmental abilities were assessed as 19 months for gross motor skills, four years for fine motor/ adaptive skills, five years for language skills and 22 months for social/ personal skills.

He had mild feeding difficulties as a baby and significant hypotonia had been noted. At the time of examination for the study, mild hypotonia was still present and his joints were mildly hypermobile. He had never had any seizures and cranial magnetic resonance imaging was reported as normal. Mild conductive hearing impairment had been noted but appeared to be resolving. Nystagmus, posterior embryotoxin, an astigmatism and decreased mixing of the optic nerve fibres had all been detected.

As a young child, he had more infections than expected but these were not unduly severe and had decreased over time.

His hair, hairlines, eyebrows and eyelashes were normal at the time of examination, although his parents commented that the hair on the top of his head did not grow in until

he was nearly one year old. Some frontal bossing was present and he had down-slanting palpebral fissures. His nose was short with a long philtrum and macrostomia. His dentition was delayed but the teeth did not look unusual. He also had a unilateral single palmar crease.

His diagnosis had been made at the age of six years on a standard lymphocyte karyotype and parental testing confirmed that his mother had a balanced chromosome translocation.

Review of the literature shows that his 1q44 deletion is likely to have an effect on his phenotype. Patients with isolated 1q44 deletions have mental retardation or learning difficulties and may have seizures, hypotonia, growth retardation, microcephaly, intracranial abnormalities and dysmorphic features[145;146]. Patients with isolated trisomy 12p also have mental retardation and may have seizures, hypotonia and dysmorphic features[147-149].

5: DISCUSSION

The literature review performed before the study commenced had confirmed that further study into Pallister-Killian syndrome was required, as most available information came from single case reports. This study has provided comprehensive data on 22 patients with Pallister-Killian syndrome. One of the benefits of a formal study is that, where possible, the absence of any specific feature is recorded as well as the rare associated features. This makes it easier to assess what proportion of patients with Pallister-Killian syndrome will have any given feature, as shown in Table 14, starting on page 123, which summarises the phenotypic data from this study in comparison to that found in the medical literature.

5a: Recruitment

It is clear from running this study that many parents of children with Pallister-Killian syndrome felt that the knowledge of the condition was inadequate and that further research was desperately needed. The families were therefore very keen to participate in the research and also to encourage others to do so, as evidenced by the two families recruited purely through other families. As previously mentioned, 15 of the 20 living patients with mosaic tetrasomy 12p (75.0%) whose families were contacted by Unique participated in the study. One of the families who did not participate later informed the researchers that they did not receive the study invitation due to a change of address. This means that a maximum of 19 families were actually contacted by Unique and the participation rate rises to 78.9% (15 out of 19) of those contacted. Even with the restricted recruitment methods imposed by the National Information Governance Board for Health and Social Care, 21 out of the possible 36 patients with Pallister-Killian syndrome living in Great Britain (58.3%) participated in the study. This is still higher than the anticipated recruitment rate of approximately 50% (see page 36) and shows how motivated the families were to find out more about this condition.

The decision of the National Information Governance Board for Health and Social Care to prevent the researchers from contacting the families via their General Practitioners obviously reduced the number of families who had the chance to participate. Equally, information from colleagues in other departments has subsequently shown that contacting the heads of Clinical Genetics departments about the research was not particularly effective, as some did not share this information with their colleagues. Some families who did not find out about the research until it had been completed have indicated that they would have taken part if they had known about the study in time. Clearly, with such small study numbers, each additional patient adds significantly to the study data.

The ages of those recruited to the study, which ranged from four months to 31 years at the time of the study interview, are broadly in keeping with the age range beyond the neonatal period seen in the literature, as shown in Figure 16 on page 67. Many (26/103; 25.2%) of those reported in the literature died in the neonatal period and this study would not have been expected to recruit this group.

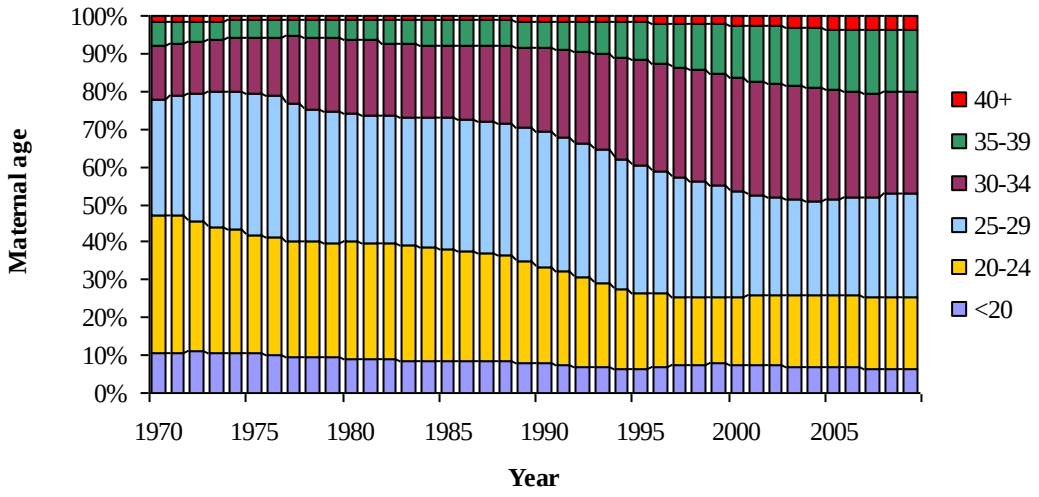
5b: Epidemiology

Prevalence

This study has confirmed that the population prevalence of Pallister-Killian syndrome in Great Britain is at least 0.6 per million, that the birth incidence is 5.1 per million (1 in just over 195,000 births) and that there were 36 patients with this condition in Great Britain in January 2010. This type of information is something families often wish to know. Many parents who participated in the study raised concerns about having taken their child to hospital, where none of the doctors had heard of Pallister-Killian syndrome. The estimates made in the preparation of this study were helpful to these families in explaining how rare the condition is. The figures calculated from the study data are more powerful than telling them the condition is rare, or that the prevalence is quoted as less than 1 per 10,000 births[1]. These figures should be helpful to families and to clinicians explaining the condition to them.

The increase in detected population prevalence, both in live-born and terminated pregnancies, is interesting. Three obvious possible reasons for this are an increased prevalence, better information regarding more recent diagnoses and better detection of the condition recently. This study and the literature[103] have both shown that there is a small increased risk for older mothers having a baby with Pallister-Killian syndrome and the average maternal age has increased over the last 40 years, as shown in Figure 42 below. However, given the small increase in risk with increasing maternal age, this is not enough to account for the increase seen in the detected population prevalence.

Figure 42: Maternal age since 1970



Some laboratories commented that their data could only go back to a certain year when a new computer system had been installed. This would reduce the population prevalence calculated using data from the earlier years and therefore give a false impression of a recent increase. Difficulties in accessing laboratory data would not, however, explain the age range of participants in the study. While these factors will have had some influence, it seems most likely that a significant part of the increase in detected population prevalence is due to better detection of the condition. It was only in 1977[2] that the underlying chromosomal anomaly in Pallister-Killian syndrome was recognised and further information about the condition has become available over time. Improvements in genetic testing, including the use of sub-telomeric MLPA analysis and the more recent introduction of array CGH testing will have increased the chance of making the diagnosis without specifically searching for Pallister-Killian syndrome.

Risk Factors

It is interesting that Pallister-Killian syndrome was found more frequently in males and that this difference was statistically significant. There is no obvious biological reason for this and, as few of the clinical features are sex-specific, it is difficult to see why the detection rate in males should be any higher than in females. Equally, there is no reason to suppose that male sex would be indicated more frequently than female sex in the information provided by the laboratories.

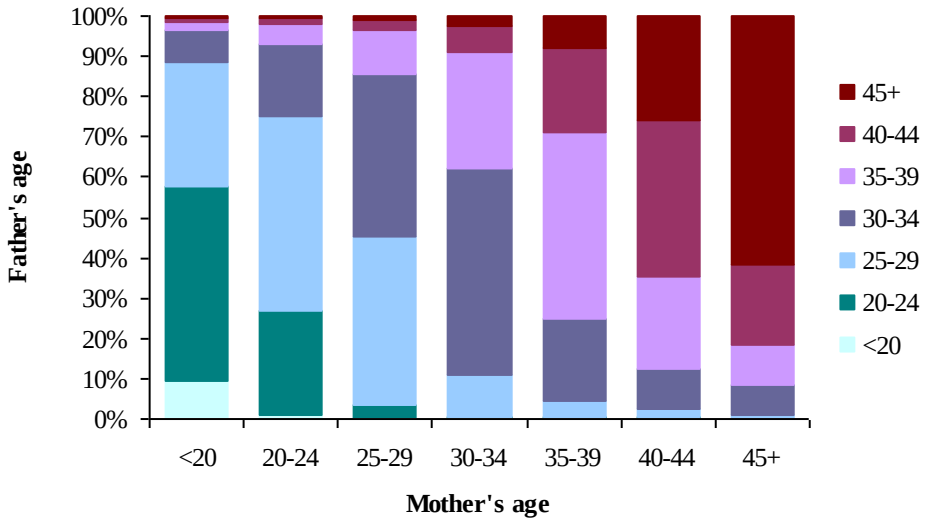
It is not possible to accurately analyse the sex ratio in the literature reports, as the population ratio is not 1:1 and the British figures cannot be extrapolated to other countries. Using the British figures as a guide, the chi-squared value for the difference between the 57 reported males and 46 reported females with Pallister-Killian syndrome due to the proven mosaic presence of an isochromosome 12p would be 0.87, giving a non-significant probability value of 0.35. However, the same analysis adding these patients to those identified in this study, gives 96 males to 62 females, with a chi-squared value of 6.14 and a probability value of 0.013.

There was no evidence from this study that assisted reproductive technology increases the risk of Pallister-Killian syndrome, as none of the recruited families had required this treatment.

This study has shown a statistically significant increase in the risk of a baby having Pallister-Killian syndrome with increasing parental age. Using maternal age data, the probability value was less than 0.001 using either the study group or all available data, while the probability values for the paternal analyses were 0.016 and 0.024 respectively. Although the data is statistically significant, the actual risk remains low, as the maximum risk calculated was an odds ratio of 8.9 (95% confidence interval of 3.4-23.0) in mothers aged at least 35 years in comparison to those aged less than 30 years.

Wenger at al[103] made the assumption that this is due to increasing maternal, rather than paternal, age but do not provide any evidence to support this, other than the fact that this has been proven in other aneuploidy conditions. Due to the small numbers involved in this study and the tendency for maternal and paternal ages to be linked, as shown in Figure 43, it is not possible to separate a maternal age effect from a paternal age effect or a combination of the two in this study either.

Figure 43: Correlation between maternal and paternal ages



This study has confirmed that the recurrence risk for Pallister-Killian syndrome is low, as documented in the literature. It is very likely that any clinical geneticist or cytogeneticist becoming aware of a recurrence within a family would seek to describe this in the medical literature, so the absence of any such reports supports this belief, as does the absence of any recurrence within the study families. The absence of any other chromosomal disorders in the siblings of the recruited patients and the absence of significant family histories of miscarriage supports this being a sporadic condition, rather than linked to an underlying parental germline anomaly. The recurrence risks to the parents and the wider family are thus low, although no definite risk figure can be calculated from the available data. Given the mild phenotypes described in some individuals in the literature and in this study, the question of recurrence risk in the offspring of Pallister-Killian patients is likely to arise in future. As this is a mosaic condition, the risk of the germ cell containing the isochromosome ranges from zero to a maximum of 50%. It is unclear whether this would reduce with age, in line with the reported age related reduction in cells containing the isochromosome in other cell lines. It is equally unclear, given the lack of understanding of the origin of the isochromosome, whether a germ cell containing an additional isochromosome could produce a viable zygote.

Mortality

Before this study commenced, there was anecdotal evidence that many patients with Pallister-Killian syndrome die at relatively young ages. The paucity of reports describing the adult phenotype and the small number of adults recruited to this study appear to support this.

Although not recorded on the death certificates viewed, those with Pallister-Killian syndrome are at increased risk of congenital heart disease and of a diaphragmatic hernia, both of which can be fatal even with the most aggressive treatment. It is also common knowledge that, in any condition causing severe mental retardation and significant congenital malformations, the mortality rate in childhood is likely to be higher than that for the general population. Families and clinicians may decide that some treatments, such as ventilation, are not in a child's best interests if the quality of life is felt to be poor. This may, in part, explain the study finding that 57.1% of the deaths recorded in Pallister-Killian syndrome were due to a respiratory infection. These could equally be due to hypotonia, poor coughing and the difficulties for a parent in knowing when a child with severe mental retardation is becoming seriously ill. This study has also shown that 54.5% of the Pallister-Killian syndrome patients had, in their parents' view, more infections than other children of a similar age and 40.9% specifically had repeated respiratory tract infections.

Although the study has shown significantly higher mortality rates in children with Pallister-Killian syndrome, it is important that these are not quoted as the risk to any given patient. It is helpful for parents of children with a mild phenotype to know that there is no evidence from this study or the literature of Pallister-Killian syndrome predisposing to childhood or adult-onset serious illnesses such as malignancies, with only one single case report mentioning a tumour[41].

5c: Phenotype and Natural History

Pallister-Killian syndrome may be detected on antenatal screening, either because of congenital malformations or a request for karyotyping due to raised maternal age and/ or an increased risk from first trimester screening procedures. We know that the results from both nuchal and serum screening can give an indication of chromosomal abnormalities other than trisomy 21[150]. A few published papers have mentioned increased nuchal translucency, both with[4;5] and without[6-9] cardiac defects, in babies subsequently found to have Pallister-Killian syndrome. The data from this study, however, shows that this is not a rare event, as at least 36.1% of antenatal diagnoses were made in the presence of an increased nuchal measurement. In at least another 8.3% of the antenatal diagnoses, biochemical serum screening had shown an increased risk of aneuploidy. However, since Pallister-Killian syndrome is so rare, this could not be used to justify mosaicism screening if a high risk from nuchal and/ or serum screening is the only documented reason for prenatal karyotyping.

Polyhydramnios is believed to be common in pregnancies where the fetus has Pallister-Killian syndrome but was only diagnosed in 17.4% of the study cases and 6.7% of cases where the laboratories provided data on terminated pregnancies. Although it was unusually severe in two of the study group and necessitated delivery at 35 weeks gestation, amnioreduction was not required in any of the mothers in the study group. Polyhydramnios in the presence of other features suggestive of Pallister-Killian syndrome may lead to mosaicism screening on prenatal testing but it would be difficult to justify it otherwise. As most of these pregnancies were not complicated by polyhydramnios, clinicians should not consider its absence to decrease the chance of the diagnosis being Pallister-Killian syndrome where other features are suggestive.

The commonest congenital malformation recorded was a diaphragmatic hernia, which fits with the literature reports. It was present in 34.8% of terminated pregnancies but not in any of the live-born patients. In many cases parents chose to terminate the pregnancy because of the congenital malformations, with the diagnosis of Pallister-Killian syndrome being made at a later date. Again, the absence of a diaphragmatic hernia should not deter clinicians from considering Pallister-Killian syndrome.

Other commonly reported congenital anomalies seen in Pallister-Killian syndrome include anal atresia[10;12;31;32;43;44;59;62;65-68] and cardiac defects[4;5;12;21;26;27;31;35;45-58]. Anal atresia was only seen in one (2.2%) of the terminated pregnancies, although two (9.1%) of the study group were known to have an anteriorly placed anus. Likewise, no serious cardiac defects were seen in the study group, with mild cardiac anomalies being detected in 18.1%.

This study has shown that numerous different congenital malformations may be detected in fetuses with Pallister-Killian syndrome. It is, however, important to remember that the antenatal ultrasound scans were normal in 65.2% of the study patients and that no significant congenital anomalies were detected antenatally in any of the study group. None of the detected congenital malformations are specific for the condition and many are just soft markers of a chromosomal abnormality. The rate of detection of congenital malformations was much higher in the groups diagnosed antenatally or terminated before the diagnosis was made than in those diagnosed postnatally, which is unsurprising. The presence of characteristic anomalies would lead to an offer of karyotyping and many laboratories would routinely perform mosaicism screening when a diaphragmatic hernia was reported.

Most of the minor congenital anomalies detected in the study group would not be detected on antenatal screening. These included cryptorchidism, inguinal hernias, umbilical hernias, the presence of only 11 pairs of ribs, a triphalangeal thumb and unilateral arrested post-axial polydactyly.

It is interesting that decreased or late fetal movements were reported in 34.8% of the study cases. In some cases this was a retrospective comparison with the patient's younger siblings and therefore would not have raised concerns during the pregnancy. This may be related to the hypotonia present in almost all neonates with Pallister-Killian syndrome. The study findings that 77.3% had significant hypotonia and another 13.6% had mild hypotonia is in keeping with the information in the literature, where hypotonia has been recorded in 98.2% of reports that mention tone. As would be expected, some of those with significant neonatal hypotonia had congenital dislocation of the hip and/ or developed a muscular scoliosis. Likewise, feeding difficulties were seen in 66.7% of the study group, with these being significant in 27.3%. These are all useful features to

consider in a patient's history but the absence of them could not be used to exclude the diagnosis.

The progression of the neurological abnormalities in Pallister-Killian is variable, with some children remaining significantly hypotonic and some later becoming spastic. Hypertonia has been reported in most of the adult patients reported[2;25;37] but was not seen in either of the adults in this study. It was, however present in an 11 year old, who had been significantly hypotonic as a neonate. Both of the patients reported by Saito et al[25] had lost motor skills during adulthood but there was no evidence of skill loss in this study and, conversely, parents reported slow progress in skill development throughout life.

The seizure phenotype reported in this study is very different from that recorded in the literature. In part, this is because many case reports have not documented the type of seizure seen. There have only been two papers in the medical literature mentioning Pallister-Killian patients with myoclonic seizures[21;29] but these were seen in 40.9% of the study patients. Only 22.7% of study group had generalised tonic-clonic seizures, the most commonly identified type of seizure in the literature. Various other seizure types, including absence seizures, late-onset infantile spasms, tonic seizures, complex partial seizures were also seen. Although the presence of myoclonic seizures may help towards a diagnosis of Pallister-Killian syndrome in the presence of other suggestive features, any type of epilepsy can be seen in this condition. The literature reports seizures commencing any time from the neonatal period[25-27] through to adulthood[28]. It is interesting that the onset of seizures seen in this study clustered between six months and the fourth birthday or aged eight to 11 years, with alteration of the seizure phenotype during puberty reported in one third of those who had completed puberty. As shown in Figure 27, the study data suggests that most patients with Pallister-Killian syndrome will develop seizures at some point in their lives.

Subtle abnormalities have been reported on cranial imaging, both in the literature and in this study. These were mainly agenesis or hypoplasia of the corpus callosum, delayed myelination[8;33;34] and mild ventriculomegaly, which may be detected antenatally. None of the study group required insertion of a shunt and this has only been reported in two cases in the literature[29;37].

Other neurological features seen in the study group included self-stimulatory behaviour in 45.5% and self-harming behaviour in 36.4%. This is a common finding in children with severe developmental delay and is not specific to Pallister-Killian syndrome. Autistic features, seen in 27.3%, are also relatively common in children with developmental delay, regardless of the underlying cause. Episodes of hyperventilation were noted in 22.7% and 40.0% of these patients also had episodes of apparent breath holding. This, and the finding that 40.9% had either anhydrosis or hypohydrosis, suggests involvement of the autonomic nervous system in Pallister-Killian syndrome. It is interesting that the rate of anhydrosis or hypohydrosis is so high, as no mention of these features was found in the medical literature.

Hearing impairment has been reported in 25.0% of the literature cases, with normal hearing recorded in only 5.3%. This study suggests this is likely to be an under-representation as hearing impairment was present in 77.3% of cases. Sensorineural, mixed and conductive hearing impairment were all seen and 31.8% required hearing aids. This data shows how important it is to formally assess hearing in children with chromosomal disorders, as learning difficulties will be worsened if hearing is impaired. Similarly, visual difficulties are common (77.3%) and should be sought. Myopia, hypermetropia, astigmatisms and squints, which are all remediable, were commonly seen.

Dysmorphology

Many of the dysmorphology findings in the study were comparable to those reported in the literature. The absence of hair in the fronto-temporal regions, seen in all of the study patients as babies and gradually improving with age, is well documented. As reported in this study but rarely mentioned in the literature, the previously bald areas may only develop sparse hair, hairs that only grow to a few centimetres in length or hair that is unusually wiry. Discrete areas of complete alopecia in other regions of the scalp were also noted in older patients. These features should be sought when considering a diagnosis of Pallister-Killian syndrome in an older patient. Reviewing photographs of patients as babies may be helpful in suggesting the diagnosis, especially in cases with a milder phenotype.

As reported in the literature, the dysmorphological phenotype progresses with time. Sparse eyebrows were common in young children but thick eyebrows were more common in the older age groups. Likewise, micrognathia was present in all of those aged less than two years but prognathia was present in both of the adults. Coarse facial features also became more prevalent with increasing age. The progression of facial features and the variability of those seen in each individual make it clear that neither the presence nor absence of any specific facial feature is definitive.

There were some facial characteristics that were present in the majority of patients and are not age-dependent. These would be helpful when considering the diagnosis. They included the short nose seen in 95.2%, long philtrum (71.4%), exaggerated cupid's bow to the mouth (57.1%) and low-set ears (57.1%). None of these features is, however, present in every individual and it is generally the gestalt, rather than a specific feature that suggests the diagnosis. Given the changing facial phenotype with age, photographs from a younger age should be reviewed wherever possible.

As formal intelligence quotient measurements were not made, the patients cannot be truly split into mild, moderate, severe and profound mental retardation groups. The developmental assessments do, however, give a good indication of these divisions. It is not clear why those with more severe phenotypes had more pronounced dysmorphic features, as there was no obvious correlation between genotype and phenotype. Since the face and the brain develop at similar embryological stages, this could represent the severity of the effect on that stage of development. The severity of the neurological phenotype could have an impact on some dysmorphic features thorough an effect on muscle usage but it is difficult to see how this could affect most of the dysmorphic facial features. It is more likely that an unidentified factor or factors influences the severity of both the neurological and the dysmorphological phenotype.

It is unclear whether the finding that most Pallister-Killian patients in the study (63.6%) had some abnormalities of skin pigmentation means that most affected individuals have pigmentary abnormalities. As this is a classic feature of mosaicism, its presence may have alerted clinicians to the possibility of mosaicism and increased the likelihood of a skin biopsy being offered and the diagnosis made. This study has shown that 36.4% of those with known Pallister-Killian syndrome do not have any evidence of alteration in

skin pigment and this may be an under-estimate. It is therefore important that the absence of pigmentary abnormalities does not detract from a possible diagnosis.

The presence of accessory nipples in only 40.9% patients is not surprising. Although this is a classical feature of Pallister-Killian syndrome, it is only recorded in 15.0% of cases reported in the literature. This figure is markedly lower than the study figure but this is probably explained by the absence of any documentation regarding accessory nipples in 72.0% of the literature cases.

Limb findings in the study group were inconsistent, with small numbers of patients having any one finding. There was, however, a typical hand shape seen in 57.1% of patients, with short, broad palms and fingers, which has not been specifically commented upon in the literature, despite being described in a number of the phenotypic case reports. Although this is not enough to make a diagnosis, it may be helpful when Pallister-Killian syndrome is being considered in the differential diagnosis. The presence of single transverse palmar creases, whether unilateral or bilateral, may also be a clue to an underlying chromosomal diagnosis. Again, the study finding of at least one single transverse palmar crease in 47.6% of the study group is markedly higher than the 15.0% recorded in the medical literature but few reports document the presence of normal palmar creases.

Delayed bone age is a common feature of chromosomal disorders so it is unsurprising that delayed closure of the anterior fontanelle and delayed eruption of the primary dentition were frequent findings. Like the other features, this may give a clue towards the diagnosis but is not specific to Pallister-Killian syndrome.

Growth

Geneticists talking about Pallister-Killian syndrome often comment that affected individuals are large babies. It is interesting, therefore, to note that none of the study group had birth weights above the 99.6th centile on the 1996 UK cross-sectional growth charts. From the literature review, only 20.0% of those reported in the neonatal period and 13.2% of those reported beyond this stage had recorded high birth weights, while 6.7% of those reported in the neonatal period had intra-uterine growth retardation. The birth weight, height and head circumference curves for those with Pallister-Killian

syndrome do seem to be shifted towards the right in comparison with the population norms, although the data is not statistically significant. While this is an interesting finding, it is clear that a low birth weight does not exclude a diagnosis of Pallister-Killian syndrome and that the majority of these babies have birth measurements within the normal ranges.

There is clear evidence of reduced growth as children with Pallister-Killian syndrome age, with the length/ height curve moving to the left of the population curve by approximately two years and then progressing further to the left with age. Similarly, the weight and head circumference curves have moved to the left of their population norms by five years and 10 years respectively. It is important to note that head circumferences are rarely documented in child health records at this age and the data was therefore too scarce to analyse properly, so the head circumferences curves may have moved to the left of the population norm anywhere between two and 10 years. Although the study data has shown clear evidence of a postnatal decline in growth velocity, it does not explain why this happens. Since the finding was of a general decline in postnatal growth velocity in the study group, rather than failure to thrive in specific individuals, it is unlikely that endocrinological investigations of growth were performed. However, as this finding only became clear on data analysis and had not been previously been reported as a feature of Pallister-Killian syndrome, no specific questions were asked of the families in this regard.

Development

One of the most interesting aspects of the study was the variability of the phenotype. Reading the literature and text books gives a picture of Pallister-Killian syndrome causing severe mental retardation, with the majority of affected individuals never learning to walk or talk. Only four (3.7%) of the 107 live-born patients reported in the medical literature, who had mosaic tetrasomy and/ or hexasomy for 12p, are documented to have mild or moderate mental retardation reported[18;20;23;24]. As Clinical Geneticists are keen to report unusual findings, you would expect those with mild or moderate mental retardation to represent less than 3.7% of an unselected cohort of those diagnosed with Pallister-Killian syndrome. This study, however, found that at least six patients (27.3%) only had mild or moderate mental retardation and three children were

coping in mainstream schooling. This is important information for families told that a fetus or a young child has Pallister-Killian syndrome.

As expected from the literature, most of the Pallister-Killian patients had some degree of developmental delay. The only exception was a four month-old baby and it is difficult to detect subtle problems at that stage. As the graphs in Figure 25 and Figure 26 show, most children had global developmental delay, often with noticeable differences between the different developmental fields (gross motor, fine motor/ adaptive, language and social/ personal). Some patients were using a form of sign language to communicate but this is not taken into account by the Denver II developmental screening test analysis, so communication skills may be under-estimated. No one developmental field was consistently better or worse across the study group.

This study has proven that many patients with Pallister-Killian syndrome do learn to walk, with more than half (53.8%) walking by the fourth birthday. The fact that one child began walking at eight years of age proves both that development progresses, albeit slowly, and that continued therapy is essential to allow these children to reach their potential. Along the same lines, one patient is reported to have completed toilet training at the age of 18 years.

Developmental assessments on each of the study patients have clarified the variability of the phenotype. Although many children did learn to walk, three children aged 10 or 11 years were unable to do so. These children had significant delay in other developmental areas, with none having any developmental skills that had progressed beyond the level expected at six months. It is difficult to correlate these findings with those of the three children with the same disorder coping in mainstream education.

5d: Genotype

All Pallister-Killian syndrome patients recruited to this study had this condition due to the mosaic presence of at least one i(12p). No other, unusual, karyotypes were detected.

As mentioned in the introduction, rare cases of mosaic hexasomy for the isochromosome 12p have been described[54;85;91]. It is interesting that this study identified one patient known to have mosaic hexasomy 12p and a second, previously known to have mosaic tetrasomy 12p who was shown to have a more complicated karyotype with mosaicism for both tetrasomy and hexasomy of 12p. Only three of the cases reported in the literature (2.8%) are known to have hexasomy 12p in comparison to the 8.7% detected in this study. It is likely that mosaic hexasomy 12p is more common than thought, as laboratory staff would have no reason to study additional cells once the diagnosis of Pallister-Killian syndrome was clear and a single cell appearing to have hexasomy 12p could easily be treated as an artefact.

Where the array CGH detected the presence of additional 12p material, it showed an increase in dosage along the full length of 12p. This supports the theory that the isochromosome in Pallister-Killian syndrome consists of two complete copies of 12p. The fact that the majority of those analysed (75.0%) had some bucccal cells with three chromosome 12 centromere FISH signals also supports the idea that a chromosome 12 centromere is present within the isochromosome. This testing does not elucidate its size and therefore this study cannot confirm that the centromere present in the isochromosome is smaller than that found in a normal chromosome 12.

The fact that array CGH detected other, unrelated array abnormalities is not surprising, as polymorphisms are relatively common in the population. It does, however, seem surprising that the rate is as high as 27.8%, as this corresponds to the estimated 25% abnormality rate found on array CGH testing in patients with previously unexplained features[134;135]. Reports show that the rate of detection of pathogenic copy number variants on array CGH testing is approximately 14%[135]. At least two of the patients (11.1%) had abnormalities other than Pallister-Killian syndrome that should cause a clinical phenotype and at least one of these was de novo. There is no obvious reason to link the development of the de novo duplication (16p13.11 to p12.3) with the formation

of an isochromosome 12p, although it seems unlikely that two rare significant genetic errors in the same patient are unrelated. In the other patient, parental samples were not analysed so it is not clear whether the deletion was inherited.

Origin Of The Isochromosome

There are two cases reported in the literature where the isochromosome 12p is likely to have resulted from a trisomy rescue[99;101]. This represents 22.2% of reported cases (2/9) where the parental origin of both 12p and 12q has been considered. If trisomy rescue was always, or usually, the cause of Pallister-Killian syndrome, uniparental disomy for 12q would be expected in approximately one third of cases and the literature data would be compatible with this. This study, however, showed bi-parental inheritance of 12q in all 11 cases where trio analysis was possible and was also able to prove this in two of the three cases where only one parent was available. This makes it unlikely that trisomy rescue is the principle cause of Pallister-Killian syndrome.

Excluding those with evidence of trisomy rescue, a further six patients in the medical literature[6;17;33;36;100] and two from this study had evidence of an additional 12p allele. In all six of the literature cases, the additional allele was of maternal origin, implying that the ovum contained additional genetic material. This could represent trisomy rescue or, alternatively, the ovum could have contained an isochromosome 12p alongside the normal copy of chromosome 12. It is unfortunate that neither of the cases with evidence of three separate alleles on 12p was informative for the parent of origin.

The final four cases in the literature where the parental origin of the additional 12p material has been considered showed an increased dosage of one allele rather than the presence of three alleles[33;36;98]. Similar findings were present in seven of the study patients. The increased dosage was in the maternal allele in three literature cases[33;36;98] and two study cases and in the paternal allele in one literature case[36] and three study cases. In these cases, the isochromosome 12p could have been present in the ovum or sperm as appropriate, an isodisomic germ cell could have provoked trisomy rescue, or the problem may have occurred in an initially normal zygote.

Although this study has provided more information on the parental origin of the isochromosome, it has not provided a definitive answer to the origin of the

isochromosome. Further studies are required, ideally in cases where skin biopsy material is still available.

5e: Genotype-Phenotype Correlation

This study has shown that those with more severe phenotypes tended to have been diagnosed at a younger age than those with mild phenotypes. This is likely to simply reflect the time at which these children were referred to Paediatric and to Genetic services. The more prominent dysmorphology noted in this group may also be relevant in allowing the diagnosis to be considered at an earlier stage.

The very variable results shown within each patient and within the fetus prove that a genotype-phenotype correlation based on the percentage of tetrasomic cells in any accessible cell-line is unlikely. Results of the analyses on three fetuses were available for this study and each of these showed a wide range of percentages of tetrasomic cells in the tissues analysed. Similarly, two skin biopsy samples that had been divided and analysed in two separate cultures showed discrepant results (17.5% compared to 82.5% in one case and 42.9% compared to 57.1% in the other).

Analyses of the buccal and skin biopsy results show no evidence of a genotype-phenotype correlation, even when taking the age at the time of sampling into consideration.

This study confirms the suggestions in the literature that there is no way to predict which children will have a milder phenotype at an early, or prenatal, stage. The array CGH and buccal mucosa FISH studies have been performed in the same laboratory using the same techniques so they are directly comparable. This is a pity, as it would be very helpful for families if a better prediction of the phenotype could be given. The most important time for this is when the diagnosis is made in the antenatal period and families are making decisions on whether or not to continue a pregnancy. It is, however, still important when dealing with young children, as parents will often consider the child's prognosis and future needs when considering having further children and making other significant long-term plans.

The lack of an obvious genotype-phenotype correlation in accessible tissues is not particularly surprising. There remains the possibility that the ratio of tetrasomic cells in other tissues, especially the brain for neurological development and the organs prone to

congenital malformations, may correlate with the severity of the phenotype. It is equally possible that it is the ratio of tetrasomic cells in each fetal tissue that is relevant and that the ratio present at birth is irrelevant. Similarly, if the isochromosome occurs as the result of trisomy rescue, it could be the site of the embryonic trisomic cells that influences the phenotype. Further possibilities include those linked to the presence of any, as yet unproven, imprinted genes on chromosome 12. As none of the patients in this study were shown to have uniparental disomy for 12q, it is not possible to assess any potential effect of this. Given the rarity of uniparental disomy for 12q shown in this study, the presence or absence of it could not fully explain the variability of the phenotype. There may also be imprinted genes on 12p, giving a parent of origin effect. Further work is required to try to elucidate any genotype-phenotype correlation.

Although it is interesting to consider the child with trisomy of the terminal portion of 12p, and he does have some features seen in Pallister-Killian syndrome, his 1q44 monosomy would be expected to influence his phenotype[145;146]. Given that the study was not aimed at patients with trisomy 12p, no real conclusions can be drawn from this single patient.

5f: Potential Methods of Diagnosis

Although Pallister-Killian syndrome is rare, it is a condition that is often considered in the differential diagnosis. It is likely that many children with Pallister-Killian syndrome, especially those without the classic features of the condition, remain undiagnosed. One of the aims of this study was to investigate whether the condition could be reliably diagnosed on array CGH testing, as this is often the first-line investigation for children with learning difficulties and/ or dysmorphic features.

The use of array CGH in prenatal samples is currently being studied in a number of regions but most laboratories are still using standard karyotypes and FISH analysis, along with additional aneuploidy screening procedures. Some antenatal diagnoses were made in the absence of any known fetal anomalies, when laboratories would not be expected to search for mosaicism. This is evidence that the isochromosome can sometimes be detected on standard prenatal karyotype preparations.

It is easy to look at the data showing that 30.8% of chorionic villus samples performed when the fetus had Pallister-Killian syndrome gave false negative results in comparison to 3.6% of amniocentesis samples and assume that the amniocentesis testing is somehow better than chorionic villus sampling, even thought the difference is not statistically significant. This may, however, reflect the data given to the laboratory at the time of the request. Chorionic villus samples are usually taken at 10- 12 weeks gestation, before most congenital anomalies would have been detected. This testing is usually performed in pregnancies at high risk of a familial disorder and is sometimes chosen where there is concern over the increased aneuploidy risk associated with maternal age. Amniocentesis samples, however, are taken from 16 weeks gestation and will be offered if congenital anomalies are detected on mid-trimester ultrasound scanning. The apparent difference between the false negative rates of the two procedures may be purely due to chance, as calculations do not show statistical significance. There are also good reasons for there to be a difference, as mentioned above, and some laboratory staff commented that they would karyotype more cells than normal, specifically looking for an isochromosome 12p, if the referral card mentioned a diaphragmatic hernia.

This study showed a low pick-up rate (16.7%) for Pallister-Killian syndrome on standard array CGH testing. To some degree this is an unexpected finding, as array CGH is carried out on DNA extracted from whole blood, thereby removing concerns regarding selection against cells containing the isochromosome during lymphocyte stimulation. Recent papers have suggested that array CGH could detect mosaicism at levels as low as 10%[112;114]. These, however, have involved additional algorithms for analysis and it is unclear whether they were run and analysed as standard samples or as a research cohort. Certainly the data from this study suggests that standard analysis fails to detect low-level mosaicism for tetrasomy 12p.

It is interesting to note that all of the patients who showed an increased dosage of 12p on the array CGH testing had tetrasomy 12p in more than half of their buccal mucosal cells. There is no evidence that the percentages of abnormal cells in the buccal mucosa and in the blood will in any way correlate but this is the only comparison available on samples taken at the same age. Comparing the three array CGH estimates of 8.5%, 22.5% and 50% with the buccal findings of 77%, 57% and 65% respectively would imply that there is no correlation between the two tissues. This is supported by the finding that one patient with 70% of buccal mucosal cells containing the isochromosome had normal array CGH results. It is unlikely that array CGH testing would have missed this level of mosaicism, as the ratio of 12p dosage to that of any other chromosomal region would be 3.4:2, which is higher than the 3:2 ration seen in non-mosaic duplications. The most likely explanation in this case is that there was a lower level of mosaicism in the lysosomes than in the buccal cells.

Equally interesting is that all of the patients with a detectable increase in 12p dosage on array CGH analysis were less than four years old. The figures of three out of six in those less than four years of age and zero out of 12 in the older age group are statistically significantly different, with a chi-squared value of 11.5 and a p value of less than 0.001. This supports the suggestion that in vivo selection causes the ratio of cells containing the isochromosome to those with a normal karyotype to decrease with age.

The array CGH samples in this study were analysed exactly as any standard NHS sample would be so it is possible that the detection rate would be higher if additional analyses were added or special attention was paid to 12p. The point of this part of the

study was to determine the effect of normal results on a standard array CGH analysis when a clinician has a suspicion of Pallister-Killian, as this is being used as the first-line chromosome analysis in many British laboratories. Despite the anecdotal and literature reports of Pallister-Killian syndrome being diagnosed following the detection of increased 12p dosage on array CGH, this study has shown that most will have normal array CGH results on standard testing. This should be a useful warning to clinicians that further investigations should be performed whenever the diagnosis is suspected.

None of those in the mild phenotype group who had array CGH testing performed would have been diagnosed by this method. This is exactly the group where the diagnosis is less likely to be suspected, as Pallister-Killian syndrome is traditionally considered a disorder causing severe mental retardation. Where the diagnosis is considered, it is helpful to know that sending a buccal sample for FISH analysis has a 75% chance of confirming the diagnosis. This applies equally to those with a mild phenotype, where four out of the five living patients had evidence of Pallister-Killian syndrome on buccal FISH analysis.

Buccal FISH analysis may be more acceptable to parents, some of whom consider a skin biopsy too invasive. It also has the benefit of being something that parents or a community nurse could do at home, or that could be performed during another clinic appointment. This could remove the requirement for an additional Clinical Genetics appointment to perform the skin biopsy.

As stated in the introduction, neither array CGH nor buccal FISH analysis in isolation can completely prove a diagnosis of Pallister-Killian syndrome, although the findings could be enough to avoid the need for a skin biopsy. If array CGH showed an increased dosage of 12p but not 12q and buccal FISH analysis showed the presence of an additional chromosome 12 centromere, the diagnosis would be clear and there would be no need for a skin biopsy. With the same array CGH findings and normal results from buccal FISH analysis, a 12p duplication, which could be in all cells or in mosaic form, or mosaicism for a supernumerary chromosome containing a single copy of 12p would remain a possibility. A simple blood karyotype would detect a non-mosaic 12p duplication. As the recurrence risk for either of the mosaic diagnoses would be low, a skin biopsy could probably be avoided. Likewise, in cases where the array CGH testing

was normal but buccal FISH analysis showed the presence of an additional chromosome 12 centromere, the recurrence risk would be low whether the diagnosis was Pallister-Killian syndrome or mosaic trisomy 12 so a skin biopsy could probably be avoided.

With current technology, karyotyping from a skin biopsy will remain the gold standard test for Pallister-Killian syndrome and will be required in many cases. It will often be performed for confirmation of diagnosis following array CGH and/ or buccal FISH analysis and will be required if these investigations do not confirm a suspected diagnosis. Where the diagnosis is strongly suspected and not confirmed on a skin biopsy, consideration should be given to performing (or repeating) buccal FISH analysis and analysing further cells from the existing skin biopsy. It has to be borne in mind that one patient in this study had a very low level of mosaicism (2%) detected in a skin biopsy sample after the review of 140 cells because mosaicism was strongly suspected. Potentially a repeat skin biopsy from a different site could be required, although this was not necessary for any of the study participants. The anecdotal cases where this has been required may represent laboratories only screening small numbers of cells for mosaicism, which could easily miss the 2% level of mosaicism mentioned above.

6: CONCLUSION

This study has met, or partially met, its aims, as shown below. In some cases, the small numbers available for study restrict the significance of the findings.

To investigate the features and natural history of Pallister-Killian syndrome in a British cohort.

This study has increased the available phenotypic information on Pallister-Killian syndrome and identified a number of interesting features of the condition. The most interesting and surprising finding was that 27.3% of the cohort only had mild or moderate mental retardation, as the medical literature describes a much poorer intellectual phenotype. Other notable findings include the frequency of myoclonic seizures, anhydrosis or hypohydrosis, hyperventilation and breath holding episodes. The progression of the dysmorphology with age has been clearly illustrated, as has the more pronounced dysmorphology associated with the more severe phenotypes.

To calculate the prevalence of the condition in live births in Great Britain.

The population prevalence of Pallister-Killian syndrome in Great Britain was calculated as at least 0.6 per million. The study clearly showed that detection of the condition is improving and the birth incidence over the five year period from 2005 to 2009 was at least 5.1 per million (1 in just over 195,000 births).

To confirm that the isochromosome consists of two whole copies of 12p.

In all cases where array CGH detected the presence of additional 12p material, it showed an increase in dosage along the full length of 12p. This confirms that, at least in those three cases, the isochromosome consisted of two whole copies of 12p.

To determine the number of different 12p alleles and their parental origin.

This study showed that some patients had had evidence of a third 12p allele but these cases were not informative for the parent of origin. More commonly, an increased dosage of one allele was seen, with this being the maternal allele in some patients and the paternal allele in others. As the results were not consistent across the 12p markers in each patient, it is difficult to draw clear conclusions from this aspect of the study.

To investigate whether or not there is a maternal or paternal age effect in this condition.

This study has shown a statistically significant increase in the risk of a baby having Pallister-Killian syndrome with increasing parental age. Due to the small numbers, it is not possible to determine whether this is due to increasing maternal age, paternal age, a combination of the two, or some other risk factor that increases with parental age.

To determine whether array CGH analysis on blood samples is sufficient to make the diagnosis, without the need for a skin biopsy.

Array CGH testing was only able to detect the increase in 12p dosage in 16.7% of the study group. All of those with positive results were less than four years old, suggesting that the diagnostic yield of this testing in Pallister-Killian syndrome decreases in older patients. Although buccal FISH analysis was positive in 75% of cases, a karyotype performed on a skin biopsy will be required in many cases where the diagnosis is suspected. Normal results from array CGH and/ or buccal FISH analysis are not enough to exclude the condition.

To investigate whether there is any link between percentage mosaicism in blood, buccal mucosa or urine samples and the severity of the condition.

This study has confirmed that there is no genotype-phenotype correlation based on the percentage of tetrasomic cells in accessible cell-lines in Pallister-Killian syndrome.

Further Research

Further research is required to determine whether simple, cost-effective alterations to array CGH analysis could improve the detection of mosaic chromosomal abnormalities such as Pallister-Killian syndrome in routine analyses. As no genotype-phenotype correlation was identified, more research is also required to elucidate the reasons for the extreme variability of the phenotype. Equally, to fully understand this condition, it is necessary to understand how and when the isochromosome develops.

7: APPENDICES

7a: Appendix 1: Details Of The Phenotype Of Liveborns With Mosaic Tetrasomy 12p Reported In The Literature

The data in Table 21 to Table 23 provides the detail from which the summary table in the Introduction (Table 4) was obtained. Each of these tables relates to the same individuals, so could be visualised as shown below.

Cases:	A1- A26	B1- B21	C1- C22	D1- D20	E1- E15
General and neurological features seen with mosaic tetrasomy 12p due to an isochromosome 12p (Table 21)	Page 180	Page 181	Page 182	Page 183	Page 184
Dysmorphology seen with (Table 22)	Page 185	Page 186	Page 187	Page 188	Page 189
Thoracic, abdominal and skeletal features seen with (Table 23)	Page 190	Page 191	Page 192	Page 193	Page 194

Table 24 the provides data on the phenotype of 10 cases reported together by Mathieu et al[52], as individual phenotypes were not reported. The cases in Table 25 were reported as having Pallister-Killian syndrome before the cytogenetic basis of the condition was established and no later report confirming or refuting the diagnosis has been identified. Patients with more atypical karyotypes who were reported as having Pallister-Killian syndrome are phenotyped in Table 27, with the exact karyotype documented in Table 26.

Table 21: General and neurological features seen with mosaic tetrasomy 12p due to an isochromosome 12p

	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14	A15	A16	A17	A18	A19	A20	A21	A22	A23	A24	A25	A26
Sex and reference(s)	F[50]	M[80]	M[13]	M[13]	F[13]	M[64]	F[70]	M[43]	F[40]	F[59]	F[66]	F[69]	F[10]	F[151]	F[63]	M[152]	F[14]	M[42]	M[44]	M[68]	M[32]	M[10]	F[60]	M[10]	F[34]	M[73]
	The cases shown on this page ('A') all died in the neonatal period																									
Polyhydramnios	+				+	+	+		+	+		+	+	+			+	+	+	+		+	+	+	+	+
Birth weight: high (H) or IUGR (R)	-	-	H	-	R		H	H	-	-	-	-	-		-		R	H	-	-	-	+	-	-	-	-
Macrocephaly (>) or microcephaly (<) at birth	-	-					>		>	-			-					>		-	-	>		-	-	-
Birth length: high (>) or low (<)	-			-			-		-	-			-					-		-		-	-	-	-	-
Feeding difficulties																					+					
Failure to thrive																										
Hypotonia as infant																		+								
Seizures																										
Microcephaly (<) or macrocephaly (>)	-						>			-			-					-			-	>		-		
Ventriculomegaly/ atrophy	-		-						-				+							-	+	+		+	+	
Other intracranial abnormalities	-		-						+				+							-	+	+		+	+	
Hearing impairment																										
Visual impairment																										
Nystagmus																										
Optic nerve atrophy/ hypoplasia																										
Retinal pallor (M= pigmentary mosaicism)																										
Hypopigmented lesions											-							+								
Hyperpigmented lesions											-															
Lymphoedema										+																
Redundant/ lax skin			-	-	-				+	+	+	+					+		+			+	+	+		+
Areas of hypertrichosis													+													

	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13	B14	B15	B16	B17	B18	B19	B20	B21
Sex and reference(s)	M[9]	F[32]	M[51]	M[12]	M[153]	M[77]	F[47]	M[78]	M[154]	M[49]	F[46]	M[47]	M[48]	F[17]	?[65]	F[19]	M[47]	F[62]	F[31]	M[45]	M[86]
Age when (last) reported (m= months; D= day)	D1	<1m	<1m	1m	5m	6m	8m	8m	9m	10m	12m	13m	13m	15m	15m	15m	17m	17m	18m	18m	18m
Polyhydramnios	+			+								+						+		+	
Birth weight: high (H) or IUGR (R)	-	-	H	-	-		-			-	-		-	-	-	H	-	-	H	-	H
Macrocephaly (>) or microcephaly (<) at birth	-	-		>	-					-				-	-	>	-	-	>	-	-
Birth length: high (>) or low (<)	-			-	-					-				>	-	>		-	>	-	-
Feeding difficulties		-		+			+				+								-		
Failure to thrive		-													+			+	-		
Hypotonia as infant	+		+			+	+		+	+	+	+	+	+	+	+	+	+	+		+
Later hypertonia																					
Mental retardation: profound (P)/ severe (S)/ mild (M)			S			+	+		+	+	+	S	+		+	+	P	+	S	P	+
Absent speech																			+		
Seizures			+								-	-		+			+		-		+
Microcephaly (<) or macrocephaly (>)		-					-		-	-	>	-	-	-	<	-	-	<	-	<	<
Ventriculomegaly/ atrophy	+	-	+	+				+	+	+	+	+	-					+	+		-
Other intracranial abnormalities		+							-		-	-	-						+		-
Hearing impairment									+			+	+						+	+	+
Visual impairment						+															+
Nystagmus						+														+	
Optic nerve atrophy/ hypoplasia																					-
Retinal pallor (M= pigmentary mosaicism)						+													+		-
Hypopigmented lesions					+	-	-	+		-		+	+	+	-		-		+	+	+
Hyperpigmented lesions						-	-			-	+			+	-		-				+
Lymphoedema			+	+			+			+		+					+				
Redundant/ lax skin		+		+											+					+	
Areas of hypertrichosis																					

	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17	C18	C19	C20	C21	C22
Sex and reference(s)	M[30]	M[155]	M[19]	F[27]	M[20]	M[26;29]	F[156]	M[29]	F[105]	M[8]	F[58]	M[53]	F[47]	M[61]	F[19]	M[87]	M[75]	F[47]	F[9]	M[72]	F[56]	F[81]
Age when (last) reported (m= months)	19m	20m	21m	21m	22m	23m	1	2	2	2	2	2	2	2	2	2	2	3	3	3	3	3
Polyhydramnios						+							+					+				
Birth weight: high (H) or IUGR (R)	H	H	-	-	-	-		H			-		H		-		-	-	-	-		H
Macrocephaly (>) or microcephaly (<) at birth			-	-	>	-					-											
Birth length: high (>) or low (<)		-	-	-	>			-			-		-		-			-		-		-
Feeding difficulties											+											
Failure to thrive			-								+	+								+		
Hypotonia as infant	+	+	+	+	+		+	+			+	+	+					+	+	+	+	
Later hypertonia																						
Mental retardation: profound (P)/ severe (S)/ mild (M)	+	+	+	S	+		P	+		+	S		+		+	S	+	P		+	S	+
Absent speech																					+	
Seizures	+	+		+		+		+		+		+	-		+			-	+		+	
Microcephaly (<) or macrocephaly (>)	-	-	-	>		-		-	-				-		-	-	-	<		-		
Ventriculomegaly/ atrophy	+			+	+	+		+		+			-				-	-		+		
Other intracranial abnormalities	-			+	+	-		+		+			-				-	-		-		
Hearing impairment				+													+	+		-		+
Visual impairment				+																		
Nystagmus	+																			+		
Optic nerve atrophy/ hypoplasia						-								+				+				
Retinal pallor (M= pigmentary mosaicism)						-								M								
Hypopigmented lesions	+		+	+				+	+			+	+	+	-		+	-		+		+
Hyperpigmented lesions					+							+	+	+			+	-				
Lymphoedema													+					+				
Redundant/ lax skin						+		+										+				
Areas of hypertrichosis																						

	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14	D15	D16	D17	D18	D19	D20
Sex and reference(s)	M[22]	M[33]	M[45]	F[157]	F[60]	F[47]	F[33]	M[33]	M[158]	M[70]	M[89;159]	M[21]	M[21]	M[47]	M[20]	F[60]	M[23;98]	F[20;79;159]	F[70]	F[20]
Age when (last) reported	4	4	4	4	4	5	5	5	5	6	6	6	6	7	7	7	8	8	9	10
Polyhydramnios	+					+												+		
Birth weight: high (H) or IUGR (R)		-	-	-	-	-	-	-		-	-	-	-	-		-		-	-	
Macrocephaly (>) or microcephaly (<) at birth		-	-	-				-			-	-	-					-		
Birth length: high (>) or low (<)	<	-	-	-			-	-			-	-	>					-	-	
Feeding difficulties				+					+									+		+
Failure to thrive		-					-	-												
Hypotonia as infant	+		+	+	+	+			+	+	+	+	+	+			+	+	+	
Later hypertonia																+				+
Mental retardation: profound (P)/ severe (S)/ mild (M)	+	P		+	+	+	P	P	+	+	S	S	S	+	M	+	M	S	+	S
Absent speech	+			+	-						+	+		+	-	-	-	+		
Seizures	+	+		+	+	+	+	+		+	+	+	+	+			+		+	+
Microcephaly (<) or macrocephaly (>)		-	-	>	-	-		-	-	-	<					<	-	>	-	<
Ventriculomegaly/ atrophy	+	+		+		-	+	+		+	+	+	-				-		+	+
Other intracranial abnormalities		+				-						-	-				-			
Hearing impairment										+	-	+			+		+	+		
Visual impairment			+			+														
Nystagmus	+					+														
Optic nerve atrophy/ hypoplasia																		-		
Retinal pallor (M= pigmentary mosaicism)																		+		
Hypopigmented lesions	+		+			+		+	+	+	+			+	+		+	+	+	+
Hyperpigmented lesions						+		+			+	+		+		+	+			+
Lymphoedema						+						+								
Redundant/ lax skin			+	+							+	+	+	+			-	+		
Areas of hypertrichosis		+				+														

	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12	E13	E14	E15
Sex and reference(s)	F[8]	M[38;88;159]	F[74]	F[24]	M[18]	F[41]	M[37]	F[47]	F[2]	F[37]	M[28]	F[25]	M[47]	M[25]	M[2;47]
Age when (last) reported (m= months)	11	11	14	14	15	15	16	18	19	21	36	37	40	43	45
Polyhydramnios		+				+									
Birth weight: high (H) or IUGR (R)	-	-	-	-	H	-	-	H	-	-		-		-	-
Macrocephaly (>) or microcephaly (<) at birth				-	-		-								
Birth length: high (>) or low (<)		-	-	-	>		-	-		-					
Feeding difficulties		+			+	+						+		+	
Failure to thrive						+									
Hypotonia as infant	+	+	+	+		+	+	+		+	-	+	+	+	+
Later hypertonia							+			+	+			+	
Mental retardation: profound (P)/ severe (S)/ mild (M)	+	P	S	M	M	+	+	+	P	S	P	+	+	+	P
Absent speech		+	+	-		+		+	+	+	+		+		+
Seizures	+	+	+	-	-	+	+	+	+		+	+	+	+	+
Microcephaly (<) or macrocephaly (>)		-	>	-	-	-	>	-	<		-		>		-
Ventriculomegaly/ atrophy	+	+	+	-		+						+		+	
Other intracranial abnormalities	+			-		+						+		+	
Hearing impairment	+	-			-		+								
Visual impairment	+														
Nystagmus						-									
Optic nerve atrophy/ hypoplasia						-	+								
Retinal pallor (M= pigmentary mosaicism)						-									
Hypopigmented lesions		+	+	+				+	+		-	+	+		+
Hyperpigmented lesions			+	+	+			+			-		+		+
Lymphoedema															-
Redundant/ lax skin		-										+		+	
Areas of hypertrichosis									+						+

Table 22: Dysmorphology seen with mosaic tetrasomy 12p due to an isochromosome 12p

	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14	A15	A16	A17	A18	A19	A20	A21	A22	A23	A24	A25	A26
Prominent forehead			+					+	+					+				+				+				
Sparse hair/ areas of alopecia		-	+	+	+			+	+	+											+					
Sparse eyebrows (or synophrys= S)			+																		+					
Sparse eyelashes																					+					
Hypertelorism/ telecanthus	+	-	+		+		+	+	+	+			+	+				+	+	+	+	+		+		+
Up-slanting palpebral fissures			-						-									+								+
Short palpebral fissures		+																				+		+	+	
Epicanthic folds					+								+				+					+		+		
Ptosis																										
Cataracts (corneal clouding= C)		C							C																C	
Flat nasal bridge	+		+		+				+	+		+					+	+	+							+
Broad nasal bridge	+		+		+			+	+	+											+					+
Short nose	+									+			+				+	+				+		+	+	
Anteverted nares	+		+						+				+				+	+	+			+		+		
Full cheeks		+																								
Long philtrum		-			+					+			+					+			+	+		+		
Macrostomia																		+	+							+
Cupid’s bow appearance to mouth			+									+														
Cleft lip (L), palate (P) or uvula (U)			-	-	-			P	P	P	LP		-				-	-				-		-	U	P
High arched palate				+	+								+				+	+			+	+			+	
Macroglossia	+								+																	
Micrognathia (M) or Prognathia (P)			M														M	M	M							
Low-set ears	+	+		+			+	+	+	+			+	+			+	+	+			+				+
Posteriorly rotated ears	+			+					+			+					+									
Small (S) or large (L) ears		S		S						S	S						S		S	S				S		S
Coarse face		+						+	+				+		+							+		+		+

	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13	B14	B15	B16	B17	B18	B19	B20	B21
Prominent forehead			+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+
Sparse hair/ areas of alopecia	+	+	+		+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+
Sparse eyebrows (or synophrys= S)		+					+				+	+	+		+		+	+			
Sparse eyelashes													+		+						
Hypertelorism/ telecanthus		+	+	+	+	+	+		+	+	+	+		+	+	+	+	+	+	+	+
Up-slanting palpebral fissures	-		+		-						+	+			-				-		
Short palpebral fissures			+												+					+	
Epicanthic folds							+	+		+		+			+		+	+	-		
Ptosis						-					+									+	
Cataracts (corneal clouding= C)											-										+
Flat nasal bridge				+	+	+		+		+	+	+	+	+	+			+		+	
Broad nasal bridge			+				+					+	+				+				-
Short nose			+	+	+						+			+	+						+
Anteverted nares			+	+	+					+	+	+	+	+	+	+			-	+	+
Full cheeks					+						+		+		+				+		+
Long philtrum			+		+			+			+	+	+		+		+		+	+	+
Macrostomia							+					+	+				+	+		+	+
Cupid’s bow appearance to mouth											+			+							
Cleft lip (L), palate (P) or uvula (U)				-									P		U			P		U	P
High arched palate	+			+					+	+					+						
Macroglossia							+						+						+		+
Micrognathia (M) or Prognathia (P)	M										P	M						M		M	
Low-set ears	+		+	+		+		+					+		+		+	+	+	+	
Posteriorly rotated ears	+			+			+	+							+		+	+		+	+
Small (S) or large (L) ears			-	S		S				S	L				S				S		
Coarse face							+				+	+	+		+		+	+	+		+

	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17	C18	C19	C20	C21	C22
Prominent forehead	+	+	+	+		+			+		+	+	+		+		+	+			+	+
Sparse hair/ areas of alopecia	+	+	+	+	+	+		+	+	+	+	+	+		+	+	+	+		+	+	+
Sparse eyebrows (or synophrys= S)	+		+	+	+					+	+	+	+					+				+
Sparse eyelashes					+																	
Hypertelorism/ telecanthus	+	+		+	+	+		+			+	+	+	+	+			+		+	+	+
Up-slanting palpebral fissures	+					+	+	-		+		-				+		-		+		+
Short palpebral fissures													+		+							
Epicanthic folds						-			+			+	+			+	+	+		+		-
Ptosis		+														+	+					
Cataracts (corneal clouding= C)						-																
Flat nasal bridge	+	+				+		+			+	+								+	+	+
Broad nasal bridge				+		-			+			+	+					+		+		
Short nose			+			+	+	+	+			-				+					+	
Anteverted nares		+	+	+	+	+	+	+	+	+	+	+				+		+		+	+	+
Full cheeks	+					+					+	+								+		-
Long philtrum	-		+		+	+		+		+					+	+						+
Macrostomia						+							+									
Cupid’s bow appearance to mouth						+		+		+										+		+
Cleft lip (L), palate (P) or uvula (U)					-	-						-		P		U						
High arched palate					+	+		+			+	+					+	+		+		
Macroglossia		+					+						+				+	+				
Micrognathia (M) or Prognathia (P)						M										M						
Low-set ears		+				+		+			+		-			+	+	+		+		
Posteriorly rotated ears						+			+		+		+					-				
Small (S) or large (L) ears													-					S				
Coarse face		+	+	+									+				+	+		+		

	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14	D15	D16	D17	D18	D19	D20
Prominent forehead	+	+	+		+	+	+	+	+	+	+	+	+	+		+	+	+	+	
Sparse hair/ areas of alopecia	+	+	+	+		+	+	+	+		+	+		+			+	+	+	+
Sparse eyebrows (or synophrys= S)	+					+	S				+	+	+				+	+		
Sparse eyelashes						-					+						-	+		
Hypertelorism/ telecanthus		+		+	+	-					+	+	+	+		+	+	+	+	
Up-slanting palpebral fissures	+	+				-	+	+	-	+	+	-	+	-				-	+	
Short palpebral fissures		+										+								
Epicanthic folds		+				+	+	+	+		+	+	+		+	+	-			
Ptosis						+					+	+								
Cataracts (corneal clouding= C)	C																-			
Flat nasal bridge	+				+	-				+	+	+				+	+	+	+	+
Broad nasal bridge				+		+				+	+	+	+				+	+		
Short nose	+		+			+				+	+		+				-	+		
Anteverted nares	+		+	+							+	+	+	+			+	+		+
Full cheeks	+		+	+	+	+														
Long philtrum						+				+	+	+	+				-	+	+	
Macrostomia	+		+	+	+	+				+	+						-		+	
Cupid’s bow appearance to mouth	+			+							+		+	+			+	+		
Cleft lip (L), palate (P) or uvula (U)	-				-					U	-				P	-	-	-		-
High arched palate	+				+						+		+	+		+		+		+
Macroglossia			+			+		+			+						-		+	
Micrognathia (M) or Prognathia (P)	P		P		M	P				P	P			M			-	-	P	
Low-set ears	+			+						+	+			+		+	-	+		
Posteriorly rotated ears											+			+			-	+		
Small (S) or large (L) ears			L				S				S	-	S	L			-	S		
Coarse face						+					+	+					-			+

	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12	E13	E14	E15
Prominent forehead	+	+			+		+	+		+	+	+	+	+	+
Sparse hair/ areas of alopecia	+	+		+	-	+	+					+	+	+	+
Sparse eyebrows (or synophrys= S)		-		+	+	+	+	+	S			+	+	+	S
Sparse eyelashes		-		+											+
Hypertelorism/ telecanthus		+	+			+	+	+	+	+	+	+	-	+	+
Up-slanting palpebral fissures		+							+				-		+
Short palpebral fissures												+		+	
Epicanthic folds			+	+	-	+		+	+				+		+
Ptosis		+													
Cataracts (corneal clouding= C)						-			+		-				+
Flat nasal bridge		+										+		+	
Broad nasal bridge	+	+			+	+	+	+	+	+	+		+		+
Short nose	+	+			+	+		+							
Anteverted nares	+	+											-		
Full cheeks		+						+				+		+	
Long philtrum		+	-			+	+	+					-		+
Macrostomia				+	+	+		+	+				+		+
Cupid’s bow appearance to mouth		+									+				
Cleft lip (L), palate (P) or uvula (U)		U			-				-						-
High arched palate		-							-			+			-
Macroglossia		+			-	+	+		+	+	+	+			+
Micrognathia (M) or Prognathia (P)	M	M	-	P	P		P	P		P			P		
Low-set ears		+	+		-	+	+					+	-		-
Posteriorly rotated ears					-								-		-
Small (S) or large (L) ears		S			-	S									L
Coarse face		+		+	-	+	+	+	+		+		+		+

Table 23: Thoracic, abdominal and skeletal features seen with mosaic tetrasomy 12p due to an isochromosome 12p

	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14	A15	A16	A17	A18	A19	A20	A21	A22	A23	A24	A25	A26
Cardiac defects	+							+	-								-	-		-	+		+		+	
Accessory nipples									-		-										+					
Diaphragmatic hernia	-	+	+	+	+		+		+	+	+	+	+	+	+	+				+		+	+	+		+
Accessory spleens	+											-			+		+			-	+	-	+			
Umbilical hernia (or exomphalos= E)								E										E	E			E				
Inguinal hernia																										
Imperforate anus			-	-	-			+		+	+								+	+	+	+				
Hypospadias								+												-		-		-		
Undescended testes				+				+										+	+	+	+	-		-		
Craniosynostosis																										
Large fontanelles/ delayed closure																						+				
Delayed eruption of teeth																										
Short neck	+		+						+	+	+	+						+	+	+					+	
Scoliosis/ kyphosis																										
<12 pairs of ribs	+																	+	+			+		+		
Short limbs: rhizomelic (R)	R			-	-							R						+	+	+	+	+		+		
Short hands and feet		-	+	-	-				+				+				+	+				+		+		
Polydactyly of fingers (pre-/post-axial= E/T)												T														
Clinodactyly																	+	+	+							
Single palmar creases: uni- (U)/ bi-lateral (B)									U	U							U									
Delayed bone age																						+				
Hypoplastic nails			+	-	-								+					+		+		+		+		
Polydactyly of toes (pre-/post-axial= E/T)	E											T					E									
Hip dislocation																										
Contractures										+											+		+			

	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13	B14	B15	B16	B17	B18	B19	B20	B21
Cardiac defects			+	+			-			+	+	+	+				+	+	+	+	-
Accessory nipples				+			+	+			-	-	+		-		-	-			
Diaphragmatic hernia																					
Accessory spleens			-																		
Umbilical hernia (or exomphalos= E)							+	+				+		+		+	+	-			
Inguinal hernia												+					+	-			
Imperforate anus		-		+											+			+	+		
Hypospadias										-											
Undescended testes				+						-		+	+							+	
Short stature								-		-			-		+			+			+
Craniosynostosis																					
Large fontanelles/ delayed closure				+			+								+						
Delayed eruption of teeth											+									+	
Short neck		+		+	+						+	+		+					+	+	
Scoliosis/ kyphosis																					
<12 pairs of ribs				+									+								
Short limbs: rhizomelic (R)												+								+	-
Short hands and feet				+				+			+	+	+	+				+	+	+	+
Polydactyly of fingers (pre-/post-axial= E/T)								T													
Clinodactyly								+			+				+						+
Single palmar creases: unilateral (U)/ bilateral (B)				U						U	B			U	B					B	
Delayed bone age																					
Hypoplastic nails				+							+	+									
Polydactyly of toes (pre-/post-axial= E/T)											E							E			
Hip dislocation														+							+
Contractures		+											+								

	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17	C18	C19	C20	C21	C22
Cardiac defects				+	+	+					+	+	-	+				-			+	
Accessory nipples								+			+	-	+			+		-				
Diaphragmatic hernia																			+			
Accessory spleens				-																		
Umbilical hernia (or exomphalos= E)						+		+					-			+		+		+		
Inguinal hernia					+									+			+					
Imperforate anus																						
Hypospadias																						
Undescended testes		+				+		+				+				+				+		
Short stature		+	-	-		-		-	+		+		-		-	-	-	+		+		
Craniosynostosis						-		+										-		+		
Large fontanelles/ delayed closure						+		+			+	+	+					+				
Delayed eruption of teeth								+										+				
Short neck			+			+					+		+									
Scoliosis/ kyphosis																		-				
<12 pairs of ribs																						
Short limbs: rhizomelic (R)																R		+		R		
Short hands and feet						+	+	+				+	+			+				+	+	
Polydactyly of fingers (pre-/post-axial= E/T)												T			+							
Clinodactyly						+																
Single palmar creases: unilateral (U)/ bilateral (B)											B					+				U		
Delayed bone age								+												+		
Hypoplastic nails						+		+														
Polydactyly of toes (pre-/post-axial= E/T)															E							
Hip dislocation													+	+				+				
Contractures											+									+		

	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14	D15	D16	D17	D18	D19	D20
Cardiac defects	-					-						+	+	-			-			
Accessory nipples		+				+								+			-	+		
Diaphragmatic hernia																				
Accessory spleens																	-			
Umbilical hernia (or exomphalos= E)	+	E			+	-					+			+				+		+
Inguinal hernia											+			+					+	
Imperforate anus (S= stenosis)		S									+						-			
Hypospadias																	-			
Undescended testes		+									+						-			
Short stature			-	-	-				+	+	+			+		-	-	+	+	+
Craniosynostosis														+						
Large fontanelles/ delayed closure					+									+		+		-		
Delayed eruption of teeth											-									
Short neck	+			+							+							+		
Scoliosis/ kyphosis											+					+				
<12 pairs of ribs																				
Short limbs: rhizomelic (R)	R																-			
Short hands and feet	+			+	+			+			+						-	+		
Polydactyly of fingers (pre-/post-axial= E/T)																				
Clinodactyly				+															+	
Single palmar creases: unilateral (U)/ bilateral (B)											-	B						B		
Delayed bone age											+							-		
Hypoplastic nails		+					+	+			-									
Polydactyly of toes (pre-/post-axial= E/T)																				
Hip dislocation											+	+	+							
Contractures																				+

	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12	E13	E14	E15
Cardiac defects						-		-	+				-		+
Accessory nipples				-				+	+	+	-		-		-
Diaphragmatic hernia															
Accessory spleens						-									
Umbilical hernia (or exomphalos= E)		-	+					-					-		-
Inguinal hernia															
Imperforate anus					-										
Hypospadias					-								-		
Undescended testes		+			-		+						-		+
Short stature				-		+	-	+	+	-	-		-		+
Craniosynostosis															
Large fontanelles/ delayed closure		-					+								
Delayed eruption of teeth	+	-													
Short neck		+			+										
Scoliosis/ kyphosis						+			+	+	+	+		+	+
<12 pairs of ribs															
Short limbs: rhizomelic (R)		-													
Short hands and feet			+												+
Polydactyly of fingers (pre-/post-axial)										+					
Clinodactyly															
Single palmar creases: unilateral (U)/ bilateral (B)		-	U						U	U					
Delayed bone age						+			+						
Hypoplastic nails															
Polydactyly of toes (pre-/post-axial= E/T)										E					
Hip dislocation		+							+						+
Contractures							+		+	+	-	+	-	+	+

Table 24: Features described by Mathieu et al[52] in a group of 10 cases

These 10 live-born individuals all have an isochromosome 12p and were reported by Mathieu et al[52]. Unfortunately they are not described individually, just as a group, so it is impossible to separate the features seen in each case and add them to the tables above.

General and neurological features	**Seen in (number) / Comment**
Polyhydramnios	
Birth weight: high (H) or IUGR (R)	10: either normal or high
Macrocephaly (>) or microcephaly (<) at birth	10: normal or macrocephaly
Birth length: high (>) or low (<)	
Feeding difficulties	
Failure to thrive	
Hypotonia as infant	10
Later hypertonia	
Mental retardation: profound (P)/ severe (S)/ mild (M)	10 (Profound)
Absent speech	10
Seizures	7
Microcephaly (<) or macrocephaly (>)	
Ventriculomegaly/ atrophy	3
Other intracranial abnormalities	
Hearing impairment	
Visual impairment	9
Nystagmus	
Optic nerve atrophy/ hypoplasia	
Retinal pallor (M= pigmentary mosaicism)	
Hypopigmented lesions	3
Hyperpigmented lesions	1
Lymphoedema	
Redundant/ lax skin	3
Areas of hypertrichosis	
Skeleton and limbs	
Short stature	
Craniosynostosis	
Large fontanelles/ delayed closure	10
Delayed eruption of teeth	
Scoliosis/ kyphosis	
<12 pairs of ribs	
Short limbs: rhizomelic (R)	4
Short hands and feet	Some or all- not clear
Polydactyly of fingers (pre-/post-axial)	
Clinodactyly	
Single palmar creases: unilateral (U)/ bilateral (B)	Some or all- not clear
Delayed bone age	
Hypoplastic nails	1
Polydactyly of toes (pre-/post-axial)	
Hip dislocation	
Contractures	

Facial features	**Seen in (number) or Comment**
Prominent forehead	Some or all- not clear
Sparse hair/ areas of alopecia	Some or all- not clear
Sparse eyebrows (or synophrys= S)	
Sparse eyelashes	
Hypertelorism/ telecanthus	Some or all- not clear
Up-slanting palpebral fissures	
Short palpebral fissures	
Epicanthic folds	
Ptosis	
Cataracts (corneal clouding= C)	
Flat nasal bridge	
Broad nasal bridge	
Short nose	Some or all- not clear
Anteverted nares	Some or all- not clear
Full cheeks	Some or all- not clear
Long philtrum	Some or all- not clear
Macrostomia	Some or all- not clear
Cupid's bow appearance to mouth	
Cleft lip (L), palate (P) or uvula (U)	
High arched palate	
Macroglossia	2
Micrognathia (M) or Prognathia (P)	M in some or all- not clear
Low-set ears	Some or all- not clear
Posteriorly rotated ears	Some
Small (S) or large (L) ears	
Coarse face	
Short neck	Some or all- not clear
Features in thorax and abdomen	
Cardiac defects	
Accessory nipples	
Diaphragmatic hernia	
Accessory spleens	
Exomphalos	
Umbilical hernia	
Inguinal hernia	
Imperforate anus	
Hypospadias	1
Undescended testes	1

Table 25: Features in those without cytogenetic confirmation of diagnosis

	F1	F2	F3	F4	F5	F6	F7	F8
Reference(s)	83;159	16;84;159	3;84;159	38;39	84;159	38;160	67	84;159
Sex	F	F	F	M	M	M	F	M
Age when (last) reported (m= months)	2	2	3	3	5	5	5	10
General and neurological features								
Polyhydramnios							+	
Birth weight: high (H) or IUGR (R)	-	H	-	-		-	-	-
Macrocephaly (>) or microcephaly (<) at birth	-	>		-	-			
Birth length: high (>) or low (<)	-	>			-			-
Feeding difficulties			+	+		+	+	+
Failure to thrive								
Hypotonia as infant	+	+	+	+	+	+	+	+
Later hypertonia								
Mental retardation: profound (P)/ severe (S)/ mild (M)	+	+	S	S	S	S	S	S
Absent speech	+	+			+			
Seizures	-	-	+		-			+
Microcephaly (<) or macrocephaly (>)	-	-			-	-	-	<
Ventriculomegaly/ atrophy	-		+	-		+		
Other intracranial abnormalities	-			+				
Hearing impairment	+		+		+			+
Visual impairment	+	-						
Nystagmus	+			-			+	
Optic nerve atrophy/ hypoplasia	-			-				
Retinal pallor (M= pigmentary mosaicism)	-			-				
Hypopigmented lesions	-	+	+		-	+		-
Hyperpigmented lesions	-	+			-			-
Lymphoedema								
Redundant/ lax skin	+	-	+	+	-			
Areas of hypertrichosis								
Skeleton and limbs								
Short stature	-	-			-	+	-	+
Craniosynostosis						-		
Large fontanelles/ delayed closure							+	
Delayed eruption of teeth	+	+	+		+			+
Scoliosis/ kyphosis	-	-	+			-		
<12 pairs of ribs								
Short limbs: rhizomelic (R)								
Short hands and feet	+	+	+	+	+	+		+
Polydactyly of fingers (pre-/post-axial)								
Clinodactyly					+	+		
Single palmar creases: unilateral (U)/ bilateral (B)	+	-	B		U	B		B
Delayed bone age	-	+	+		-			
Hypoplastic nails	+	-	-	-	-			
Polydactyly of toes (pre-/post-axial)								
Hip dislocation	-	-			+	-	+	
Contractures								

	F1	F2	F3	F4	F5	F6	F7	F8
Facial features								
Prominent forehead	+	+	+	+	-	+		-
Sparse hair/ areas of alopecia	+	+	+	+	+	+	+	+
Sparse eyebrows (or synophrys= S)	-	+	+		+			+
Sparse eyelashes	+	+	+		+			+
Hypertelorism/ telecanthus	+	+	+	+	-		+	+
Up-slanting palpebral fissures	-	+	-	+	-	+		
Short palpebral fissures							+	
Epicanthic folds			-	+		+	+	
Ptosis	+	+	+		+		+	+
Cataracts (corneal clouding= C)								
Flat nasal bridge	+	+	+	+	+			+
Broad nasal bridge	+	+	+		+			+
Short nose	+	+	+	+	+			+
Anteverted nares	+	+	+	+	+			+
Full cheeks			+	+				
Long philtrum	+	+	+		+	-	+	+
Macrostomia				+		+		
Cupid's bow appearance to mouth	+	+	+		+			+
Cleft lip (L), palate (P) or uvula (U)	-	-	U		-			-
High arched palate	+	+	+	+	-		+	+
Macroglossia		-	+		-			
Micrognathia (M) or Prognathia (P)	-	-	M	M	M			M
Low-set ears		-	+		+	-		+
Posteriorly rotated ears						-		
Small (S) or large (L) ears								
Coarse face	+							+
Short neck	+	+	+	+	+			+
Features in thorax and abdomen								
Cardiac defects								
Accessory nipples								
Diaphragmatic hernia								
Accessory spleens								
Exomphalos	-	-	-	-				-
Umbilical hernia	-	-	-	+				+
Inguinal hernia	-	-	-		+			+
Imperforate anus							+	
Hypospadias								
Undescended testes				+		+		

Table 26: Atypical karyotypes reported as Pallister-Killian syndrome

G1[91]	Mosaic 48,XX +i(12p)+i(12p)/ 46,XX
G2[15]	Mosaic 46,XY, trp(12)(p11.2-p13)
G3[78]	Mosaic 47,XX +12p/ 47,XX +i(12p)/ 46,XX
G4[95]	Mosaic for supernumerary ring chromosome containing 2 copies of 12p
G5[82]	Mosaic tetrasomy for 12pter-12p11.2
G6[85]	Mosaic 47,XX +i(12p)/ 48,XX +i(12p)+i(12p)/ 46,XX
G7[34]	Mosaic 47,XX +12p/ 47,XX +i(12p)/ 46,XX
G8[54]	Mosaic 48,XX +i(12p)+i(12p)/ 46,XX
G9[94]	Mosaic tetrasomy for 12pter-12p12.3

Table 27: Features seen in those with atypical karyotypes

	G1[91]	G2[15]	G3[78]	G4[95]	G5[82]	G6[85]	G7[34]	G8[54]	G9[94]
Sex	F	M	F	F	M	F	F	F	F
Age when (last) reported (m= months)	D1	4m	19m	2	2	2	5	5	6
General and neurological features									
Polyhydramnios	+					+		+	
Birth weight: high (H) or IUGR (R)	R	-	-	-	H	-	H	-	-
Macrocephaly (>) or microcephaly (<) at birth	<	<	>		-		-	>	-
Birth length: high (>) or low (<)	<	-	-	-	-			-	-
Feeding difficulties									
Failure to thrive		-		+		+		+	
Hypotonia as infant		+	+		+		+	+	+
Later hypertonia									
Mental retardation: profound (P)/ severe (S)/ mild (M)		+	+	+	+	+	+	M	+
Absent speech			-		+		+		+
Seizures					-	-	+		+
Microcephaly (<) or macrocephaly (>)		-	-	-	<		>	-	<
Ventriculomegaly/ atrophy			+			-	+	-	
Other intracranial abnormalities			-				+	-	
Hearing impairment					+	+		+	
Visual impairment									
Nystagmus				+	+	+			
Optic nerve atrophy/ hypoplasia									
Retinal pallor (M= pigmentary mosaicism)			+						
Hypopigmented lesions			-		+	+		+	-
Hyperpigmented lesions			-						-
Lymphoedema									
Redundant/ lax skin	+								
Areas of hypertrichosis									
Skeleton and limbs									
Short stature		+		+	-			-	-
Craniosynostosis									
Large fontanelles/ delayed closure			+						
Delayed eruption of teeth									
Scoliosis/ kyphosis									
<12 pairs of ribs									
Short limbs: rhizomelic (R)	R					R	+		
Short hands and feet				+			+		+
Polydactyly of fingers (pre-/post-axial)								E	
Clinodactyly									
Single palmar creases: unilateral (U)/ bilateral (B)		B		B	B				U
Delayed bone age									
Hypoplastic nails								+	
Polydactyly of toes (pre-/post-axial)									
Hip dislocation								+	+
Contractures	+								

	G1	G2	G3	G4	G5	G6	G7	G8	G9
Facial features									
Prominent forehead	+	+	+	+		+	+	+	+
Sparse hair/ areas of alopecia	+	+	+	+	+	+	+	+	+
Sparse eyebrows (or synophrys= S)			+				+		
Sparse eyelashes									
Hypertelorism/ telecanthus	+				+		+	+	+
Up-slanting palpebral fissures		+					+		
Short palpebral fissures									+
Epicanthic folds					+		+		
Ptosis				+					+
Cataracts (corneal clouding= C)									
Flat nasal bridge		+	+				+		+
Broad nasal bridge							+	+	+
Short nose	+	+	+				+		+
Anteverted nares					+	+	+	+	
Full cheeks		+					+		
Long philtrum	+		+	+	+	+	+	+	
Macrostomia	+		+	+			+		
Cupid's bow appearance to mouth	+								
Cleft lip (L), palate (P) or uvula (U)		-		P		P	-	-	-
High arched palate							+	+	+
Macroglossia			+				+		
Micrognathia (M) or Prognathia (P)	M							M	
Low-set ears	+				+		+	+	+
Posteriorly rotated ears			+		+				
Small (S) or large (L) ears			L				S	L	S
Coarse face	+						+		
Short neck	+						+		
Features in thorax and abdomen									
Cardiac defects	+					-		+	+
Accessory nipples				+					
Diaphragmatic hernia	+					+			
Accessory spleens						-			
Exomphalos	-					+	-		
Umbilical hernia							+		
Inguinal hernia								+	
Imperforate anus				+					
Hypospadias									
Undescended testes									

7b: Appendix 2: Parental Age Linear Regression Results

Figure 44: Results of linear regression analysis for Pallister-Killian Syndrome by maternal age in the study group

Crosstabs Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Mother's age at delivery Affected	585941	100.0%	0	.0%	585941	100.0%

Mother's age at delivery * Affected Crosstabulation

		Affected		
		No	Yes	Total
Mother's age at delivery	<20	34454	1	34455
	20-24	98699	0	98699
	25-29	182469	3	182472
	30-34	181725	6	181731
	35-39	75719	11	75730
	40-44	12306	1	12307
	45+	547	0	547
Total		585919	22	585941

Logistic Regression Case Processing Summary

Unweighted Cases		N	Percent
Selected Cases	Included in Analysis	585941	100.0
	Missing Cases	0	.0
	Total	585941	100.0
Unselected Cases		0	.0
Total		585941	100.0

Classification Table

		Predicted		
		Affected		
Observed		No	Yes	Percentage Correct
Affected	No	585919	0	100.0
	Yes	22	0	.0
Overall Percentage				100.0

Block 0: Beginning Block: Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)
Step 0	Constant	-10.190	.213	2284.261	1	.000	.000

Variables not in the Equation

			Score	df	Sig.
Step 0	Variables	Age	30.083	6	.000
		Age(1)	4.457	1	.035
		Age(2)	3.144	1	.076
		Age(3)	.144	1	.704
		Age(4)	26.872	1	.000
		Age(5)	.640	1	.424
		Age(6)	.021	1	.886
	Overall Statistics		30.083	6	.000

Block 1: Method = Enter

Omnibus Tests of Model Coefficients

		Chi-square	df	Sig.
Step 1	Step	24.296	6	.000
	Block	24.296	6	.000
	Model	24.296	6	.000

Model Summary

Step	-2 Log likelihood	Cox & Snell R Square	Nagelkerke R Square
1	468.060[a]	.000	.049

a. Estimation terminated at iteration number 20 because maximum iterations has been reached. Final solution cannot be found.

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
								Lower	Upper
Step 1[a]	Age			16.243	6	.013			
	Age(1)	-10.756	127.940	.007	1	.933	.000	.000	1.705E104
	Age(2)	-.568	1.155	.242	1	.623	.566	.059	5.446
	Age(3)	.129	1.080	.014	1	.905	1.138	.137	9.449
	Age(4)	1.610	1.044	2.377	1	.123	5.005	.646	38.770
	Age(5)	1.030	1.414	.530	1	.467	2.800	.175	44.765
	Age(6)	-10.756	1718.537	.000	1	.995	.000	.000	.
	Constant	-13.206	246.185	.003	1	.957	.000		

a. Variable(s) entered on step 1: Age.

Figure 45: Results of linear regression analysis for Pallister-Killian syndrome by maternal age in the study group with wider age bands

Crosstabs Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Mother's age at delivery Affected	585941	100.0%	0	.0%	585941	100.0%

Mother's age at delivery * Affected Crosstabulation

		Affected		
		No	Yes	Total
Mother's age at delivery	<30	315622	4	315626
	30-34	181725	6	181731
	35+	88572	12	88584
Total		585919	22	585941

Logistic Regression Case Processing Summary

Unweighted Cases		N	Percent
Selected Cases	Included in Analysis	585941	100.0
	Missing Cases	0	.0
	Total	585941	100.0
Unselected Cases		0	.0
Total		585941	100.0

Classification Table

		Predicted		
		Affected		Percentage Correct
Observed		No	Yes	
Affected	No	585919	0	100.0
	Yes	22	0	.0
Overall Percentage				100.0

Block 0: Beginning Block: Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)
Step 0	Constant	-10.190	.213	2284.261	1	.000	.000

Variables not in the Equation

			Score	df	Sig.
Step 0	Variables	Age_band	27.922	2	.000
		Age_band(1)	.144	1	.704
		Age_band(2)	26.651	1	.000
	Overall Statistics		27.922	2	.000

Block 1: Method = Enter

Omnibus Tests of Model Coefficients

		Chi-square	df	Sig.
Step 1	Step	20.564	2	.000
	Block	20.564	2	.000
	Model	20.564	2	.000

Model Summary

Step	-2 Log likelihood	Cox & Snell R Square	Nagelkerke R Square
1	471.792[a]	.000	.042

a. Estimation terminated at iteration number 14 because parameter estimates changed by less than .001.

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
								Lower	Upper
Step 1[a]	Age_band			19.771	2	.000			
	Age_band(1)	.958	.646	2.200	1	.138	2.605	.735	9.232
	Age_band(2)	2.369	.577	16.841	1	.000	10.690	3.448	33.147
	Constant	-10.167	.236	1860.567	1	.000	.000		

a. Variable(s) entered on step 1: Age_band.

Figure 46: Results of linear regression analysis for Pallister-Killian syndrome by maternal age utilising all available data

Crosstabs Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Mother's age at delivery Affected	585941	100.0%	0	.0%	585941	100.0%

Mother's age at delivery * Affected Crosstabulation

		Affected		
		No	Yes	Total
Mother's age at delivery	<20	34454	1	34455
	20-24	98698	1	98699
	25-29	182468	4	182472
	30-34	181722	9	181731
	35-39	75717	13	75730
	40-44	12305	2	12307
	45+	547	0	547
Total		585911	30	585941

Logistic Regression Case Processing Summary

Unweighted Cases		N	Percent
Selected Cases	Included in Analysis	585941	100.0
	Missing Cases	0	.0
	Total	585941	100.0
Unselected Cases		0	.0
Total		585941	100.0

Classification Table

		Predicted		
		Affected		Percentage Correct
Observed		No	Yes	
Affected	No	585911	0	100.0
	Yes	30	0	.0
Overall Percentage				100.0

Block 0: Beginning Block: Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)
Step 0	Constant	-9.880	.183	2928.120	1	.000	.000

Variables not in the Equation

			Score	df	Sig.
Step 0	Variables	Age	31.119	6	.000
		Age(1)	3.910	1	.048
		Age(2)	4.437	1	.035
		Age(3)	.014	1	.904
		Age(4)	24.651	1	.000
		Age(5)	3.042	1	.081
		Age(6)	.028	1	.867
	Overall Statistics		31.119	6	.000

Block 1: Method = Enter

Omnibus Tests of Model Coefficients

		Chi-square	df	Sig.
Step 1	Step	24.316	6	.000
	Block	24.316	6	.000
	Model	24.316	6	.000

Model Summary

Step	-2 Log likelihood	Cox & Snell R Square	Nagelkerke R Square
1	628.469[a]	.000	.037

a. Estimation terminated at iteration number 20 because maximum iterations has been reached. Final solution cannot be found.

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
								Lower	Upper
Step 1[a]	Age			22.605	6	.001			
	Age(1)	-1.052	1.414	.554	1	.457	.349	.022	5.581
	Age(2)	-.281	1.118	.063	1	.802	.755	.084	6.758
	Age(3)	.534	1.054	.257	1	.612	1.706	.216	13.469
	Age(4)	1.778	1.038	2.934	1	.087	5.915	.774	45.221
	Age(5)	1.723	1.225	1.978	1	.160	5.600	.508	61.763
	Age(6)	-10.756	1718.547	.000	1	.995	.000	.000	.
	Constant	-11.598	245.507	.002	1	.962	.000		

a. Variable(s) entered on step 1: Age.

Figure 47: Results of linear regression analysis for Pallister-Killian syndrome by maternal age utilising all available data with wider age bands

Crosstabs Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Mother's age at delivery Affected	585941	100.0%	0	.0%	585941	100.0%

Mother's age at delivery * Affected Crosstabulation

		Affected		
		No	Yes	Total
Mother's age at delivery	<30	315620	6	315626
	30-34	181722	9	181731
	35+	88569	15	88584
Total		585911	30	585941

Logistic Regression Case Processing Summary

Unweighted Cases		N	Percent
Selected Cases	Included in Analysis	585941	100.0
	Missing Cases	0	.0
	Total	585941	100.0
Unselected Cases		0	.0
Total		585941	100.0

Classification Table

		Predicted		
		Affected		Percentage Correct
Observed		No	Yes	
Affected	No	585911	0	100.0
	Yes	30	0	.0
Overall Percentage				100.0

Block 0: Beginning Block: Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)
Step 0	Constant	-9.880	.183	2928.120	1	.000	.000

Variables not in the Equation

			Score	df	Sig.
Step 0	Variables	Age_band	30.544	2	.000
		Age_band(1)	.014	1	.904
		Age_band(2)	28.446	1	.000
	Overall Statistics		30.544	2	.000

Block 1: Method = Enter

Omnibus Tests of Model Coefficients

		Chi-square	df	Sig.
Step 1	Step	23.397	2	.000
	Block	23.397	2	.000
	Model	23.397	2	.000

Model Summary

Step	-2 Log likelihood	Cox & Snell R Square	Nagelkerke R Square
1	629.388[a]	.000	.036

a. Estimation terminated at iteration number 14 because parameter estimates changed by less than .001.

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
								Lower	Upper
Step 1[a]	Age_band			22.801	2	.000			
	Age_band(1)	.958	.527	3.301	1	.069	2.605	.927	7.319
	Age_band(2)	2.187	.483	20.498	1	.000	8.909	3.457	22.962
	Constant	-9.822	.196	2520.742	1	.000	.000		

a. Variable(s) entered on step 1: Age_band.

Figure 48: Results of linear regression analysis for Pallister-Killian syndrome by paternal age in the study group

Crosstabs Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Father's age at delivery Affected	585941	100.0%	0	.0%	585941	100.0%

Father's age at delivery * Affected Crosstabulation

		Affected		
		No	Yes	Total
Father's age at delivery	<20	11326	1	11327
	20-24	60087	0	60087
	25-29	153074	2	153076
	30-34	193513	6	193519
	35-39	111478	7	111485
	40-44	38418	4	38422
	45+	18023	2	18025
Total		585919	22	585941

Logistic Regression Case Processing Summary

Unweighted Cases[a]		N	Percent
Selected Cases	Included in Analysis	585941	100.0
	Missing Cases	0	.0
	Total	585941	100.0
Unselected Cases		0	.0
Total		585941	100.0

a. If weight is in effect, see classification table for the total number of cases.

Classification Table

		Predicted		
		Affected		Percentage Correct
Observed		No	Yes	
Affected	No	585919	0	100.0
	Yes	22	0	.0
Overall Percentage				100.0

Block 0: Beginning Block: Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)
Step 0	Constant	-10.190	.213	2284.261	1	.000	.000

Variables not in the Equation

			Score	df	Sig.
Step 0	Variables	Age	14.710	6	.023
		Age(1)	2.514	1	.113
		Age(2)	3.308	1	.069
		Age(3)	.329	1	.566
		Age(4)	2.337	1	.126
		Age(5)	4.852	1	.028
		Age(6)	2.669	1	.102
	Overall Statistics		14.710	6	.023

Block 1: Method = Enter

Omnibus Tests of Model Coefficients

		Chi-square	df	Sig.
Step 1	Step	14.882	6	.021
	Block	14.882	6	.021
	Model	14.882	6	.021

Model Summary

Step	-2 Log likelihood	Cox & Snell R Square	Nagelkerke R Square
1	477.474[a]	.000	.030

a. Estimation terminated at iteration number 20 because maximum iterations has been reached. Final solution cannot be found.

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B) Lower	Upper
Step 1[a]	Age			9.027	6	.172			
	Age(1)	-11.868	163.971	.005	1	.942	.000	.000	2.621E134
	Age(2)	-1.911	1.225	2.434	1	.119	.148	.013	1.632
	Age(3)	-1.046	1.080	.939	1	.333	.351	.042	2.917
	Age(4)	-.341	1.069	.102	1	.750	.711	.087	5.781
	Age(5)	.165	1.118	.022	1	.883	1.179	.132	10.552
	Age(6)	.229	1.225	.035	1	.852	1.257	.114	13.862
	Constant	-11.445	23.425	.239	1	.625	.000		

a. Variable(s) entered on step 1: Age.

Figure 49: Results of linear regression analysis for Pallister-Killian syndrome by paternal age in the study group with wider age bands

Crosstabs Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Father's age at delivery Affected	585941	100.0%	0	.0%	585941	100.0%

Father's age at delivery * Affected Crosstabulation

		Affected		
		No	Yes	Total
Father's age at delivery	<30	224487	3	224490
	30-34	193513	6	193519
	35-39	111478	7	111485
	40+	56441	6	56447
Total		585919	22	585941

Logistic Regression Case Processing Summary

Unweighted Cases		N	Percent
Selected Cases	Included in Analysis	585941	100.0
	Missing Cases	0	.0
	Total	585941	100.0
Unselected Cases		0	.0
Total		585941	100.0

Classification Table

		Predicted		
		Affected		Percentage Correct
Observed		No	Yes	
Affected	No	585919	0	100.0
	Yes	22	0	.0
Overall Percentage				100.0

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)
Step 0	Constant	-10.190	.213	2284.261	1	.000	.000

Block 0: Beginning Block: Variables not in the Equation

			Score	df	Sig.
Step 0	Variables	Age_band	12.715	3	.005
		Age_band(1)	.329	1	.566
		Age_band(2)	2.337	1	.126
		Age_band(3)	7.863	1	.005
	Overall Statistics		12.715	3	.005

Block 1: Method = Enter

Omnibus Tests of Model Coefficients

		Chi-square	df	Sig.
Step 1	Step	11.191	3	.011
	Block	11.191	3	.011
	Model	11.191	3	.011

Model Summary

Step	-2 Log likelihood	Cox & Snell R Square	Nagelkerke R Square
1	481.165[a]	.000	.023

a. Estimation terminated at iteration number 14 because parameter estimates changed by less than .001.

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
								Lower	Upper
Step 1[a]	Age_band			10.347	3	.016			
	Age_band(1)	.842	.707	1.417	1	.234	2.320	.580	9.277
	Age_band(2)	1.547	.690	5.027	1	.025	4.699	1.215	18.171
	Age_band(3)	2.074	.707	8.601	1	.003	7.955	1.989	31.808
	Constant	-10.107	.225	2019.019	1	.000	.000		

a. Variable(s) entered on step 1: Age_band.

Figure 50: Results of linear regression analysis for Pallister-Killian syndrome by paternal age utilising all available data

Crosstabs Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Father's age at delivery Affected	585941	100.0%	0	.0%	585941	100.0%

Father's age at delivery * Affected Crosstabulation

		Affected		
		No	Yes	Total
Father's age at delivery	<20	11326	1	11327
	20-24	60086	1	60087
	25-29	153071	5	153076
	30-34	193513	6	193519
	35-39	111477	8	111485
	40-44	38418	4	38422
	45+	18022	3	18025
Total		585913	28	585941

Logistic Regression Case Processing Summary

Unweighted Cases		N	Percent
Selected Cases	Included in Analysis	585941	100.0
	Missing Cases	0	.0
	Total	585941	100.0
Unselected Cases		0	.0
Total		585941	100.0

Classification Table

		Predicted		
		Affected		Percentage Correct
Observed		No	Yes	
Affected	No	585913	0	100.0
	Yes	28	0	.0
Overall Percentage				100.0

Block 0: Beginning Block: Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)
Step 0	Constant	-9.949	.189	2771.226	1	.000	.000

Variables not in the Equation

			Score	df	Sig.
Step 0	Variables	Age	12.683	6	.048
		Age(1)	1.359	1	.244
		Age(2)	.992	1	.319
		Age(3)	1.703	1	.192
		Age(4)	1.656	1	.198
		Age(5)	2.730	1	.099
		Age(6)	5.479	1	.019
	Overall Statistics		12.683	6	.048

Block 1: Method = Enter

Omnibus Tests of Model Coefficients

		Chi-square	df	Sig.
Step 1	Step	10.344	6	.111
	Block	10.344	6	.111
	Model	10.344	6	.111

Model Summary

Step	-2 Log likelihood	Cox & Snell R Square	Nagelkerke R Square
1	602.785[a]	.000	.017

a. Estimation terminated at iteration number 14 because parameter estimates changed by less than .001.

Variables in the Equation

								95% C.I.for EXP(B)	
		B	S.E.	Wald	df	Sig.	Exp(B)	Lower	Upper
Step 1[a]	Age			10.801	6	.095			
	Age(1)	-1.669	1.414	1.392	1	.238	.188	.012	3.014
	Age(2)	-.994	1.095	.824	1	.364	.370	.043	3.167
	Age(3)	-1.046	1.080	.939	1	.333	.351	.042	2.917
	Age(4)	-.207	1.061	.038	1	.845	.813	.102	6.499
	Age(5)	.165	1.118	.022	1	.883	1.179	.132	10.552
	Age(6)	.634	1.155	.302	1	.583	1.885	.196	18.127
	Constant	-9.780	.251	1524.131	1	.000	.000		

a. Variable(s) entered on step 1: Age.

Figure 51: Results of linear regression analysis for Pallister-Killian syndrome by paternal age utilising all available data with wider age bands

Crosstabs Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Father's age at delivery Affected	585941	100.0%	0	.0%	585941	100.0%

Father's age at delivery * Affected Crosstabulation

Count

		Affected		
		No	Yes	Total
Father's age at delivery	<30	224483	7	224490
	30-34	193513	6	193519
	35-39	111477	8	111485
	40+	56440	7	56447
Total		585913	28	585941

Logistic Regression Case Processing Summary

Unweighted Cases		N	Percent
Selected Cases	Included in Analysis	585941	100.0
	Missing Cases	0	.0
	Total	585941	100.0
Unselected Cases		0	.0
Total		585941	100.0

Classification Table

			Predicted		
			Affected		Percentage Correct
	Observed		No	Yes	
Step 0	Affected	No	585913	0	100.0
		Yes	28	0	.0
	Overall Percentage				100.0

Block 0: Beginning Block: Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)
Step 0	Constant	-9.949	.189	2771.226	1	.000	.000

Variables not in the Equation

			Score	df	Sig.
Step 0	Variables	Age_band	10.640	3	.014
		Age_band(1)	1.703	1	.192
		Age_band(2)	1.656	1	.198
		Age_band(3)	7.595	1	.006
	Overall Statistics		10.640	3	.014

Block 1: Method = Enter

Omnibus Tests of Model Coefficients

		Chi-square	df	Sig.
Step 1	Step	8.688	3	.034
	Block	8.688	3	.034
	Model	8.688	3	.034

Model Summary

Step	-2 Log likelihood	Cox & Snell R Square	Nagelkerke R Square
1	604.441[a]	.000	.014

a. Estimation terminated at iteration number 13 because parameter estimates changed by less than .001.

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
								Lower	Upper
Step 1[a]	Age_band			9.413	3	.024			
	Age_band(1)	-.006	.556	.000	1	.992	.994	.334	2.959
	Age_band(2)	.834	.518	2.594	1	.107	2.301	.835	6.347
	Age_band(3)	1.381	.535	6.671	1	.010	3.977	1.395	11.340
	Constant	-9.824	.190	2674.027	1	.000	.000		

a. Variable(s) entered on step 1: Age_band.

7c: Appendix 3: Results Of Microsatellite Analysis

Table 28: Results of microsatellite analysis

	12p					12q		
	D12S94	D12S1608	D12S1694	D12S1725	D12S1692	D12S1635	D12S1598	D12S819
A	195, 199	237, 245	259, 263	162, 164	252	131, 144	162, 166	336, 340
	199	237, 247	257, 263	158, 162	failed	131, 140	162, 166	336, 343
	193, 195	245, 249	234, 259	162, 164	252, 261	144	166, 168	338
B	failed	251, 255	251, 267	160	252, 259	144, 146	failed	failed
	199, 201	failed	263, 267	146, 160	259	139, 144	166	332, 340
	195, 204	247, 251	235, 251	156, 160	249, 252	146, 148	162, 166	328, 346
C	199	252, 253	257, 261	160	250, 251	142, 144	154, 174	347
	195, 199	245, 253	257, 265	156, 160	251, 261	142, 144	154, 170	failed
	199, 203	241, 252	261, 269	160, 162	250	137, 142	160, 174	336, 346
D	189, 199	237	259, 265	160	255, 259	144, 148	162, 170	336, 350
	182, 199	237, 255	259, 265	160	259	144, 146	168, 170	343, 350
	189, 197	237	259, 263	failed	255, 259	146, 148	162, 170	336, 346

	12p					12q		
	D12S94	D12S1608	D12S1694	D12S1725	D12S1692	D12S1635	D12S1598	D12S819
E	182, 199	245, 251	257, 259, 261	152, 158	255, 257	146	162, 166	338
	189, 199	245, 253	259, 261	156, 158	251, 257	140, 146	162, 170	340, 347
	182, 203	247, 251	257, 259	152, 158	255, 261	146, 148	166	336, 340
F	197, 199	239, 245*	255, 261*	154, 162	257, 259	144, 146	162, 166	336, 347
	197	237, 239	253, 255	152, 162	253, 259	139, 146	162, 166	336
	195, 199	245, 249	251, 261	154, 160	252, 257	144	162, 164	340, 347
G	195	237	261, 263	160, 162	259	146, 148	162, 166	332, 340
	195	237, 249	261	146, 162	252, 259	146, 150	162, 168	340, 374
	195, 199	237	257, 263	158, 160	257, 259	144, 148	166	329, 332
H	195, 199	249	259, 263*	144, 146	248, 250	140, 148	162, 166	320, 347
	189, 199	245, 249	255, 263	142, 144	249, 251	135, 148	164, 166	320, 336
	195, 197	249, 251	259, 265	146, 160	251, 261	140, 144	162, 170	340, 346
I	189	235, 249	253, 261	158	250, 255	146	168	320, 332
	189, 195	249	261	158	250, 251	146	162, 168	332, 336
	189	235, 241	253	158	250, 252	146	162, 168	320, 346

	12p					12q		
	D12S94	D12S1608	D12S1694	D12S1725	D12S1692	D12S1635	D12S1598	D12S819
J	189*, 197 197 189, 199	237*, 239 239, 253 237	257*, 267 261, 267 257, 263	152 146, 152 152, 160	250 244, 250 250, 254	139 139, 148 139, 148	158, 171 169, 171 158, 166	325, 336 324, 343 332, 336
K	197, 199 195, 199 197, 199	234, 249* 234, 249 237, 249	263, 267 263 255, 267	152, 158 152, 158 158	250, 261 257, 261 250, 252	148, 150 146, 148 140, 150	failed 162, 168 168, 170	340 340 320, 340
L	193, 199 199	245, 253 245, 247	257, 259 257, 263	146, 158 152, 158	259 259	150, 152 148, 152	162, 169 162, 164	343, 353 340, 343
M	189, 197* 197, 199	237, 245* 245	235, 265* 235, 253	146, 174 162, 174	257, 261* 261	139, 148 139, 148	172 170, 172	338, 350 338, 350
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