Whole genome analysis as a diagnostic tool for patients referred for diagnosis of Silver-Russell syndrome – a real-world study.

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ABSTRACT

Background: Silver-Russell syndrome (SRS) is an imprinting disorder characterised by prenatal and postnatal growth restriction, but its clinical features are nonspecific and its differential diagnosis is broad. Known molecular causes of SRS include imprinting disturbance, single nucleotide variants, copy number variants or uniparental disomy affecting several genes; but up to 40% of individuals with a clinical diagnosis of SRS currently receive no positive molecular diagnosis.

Methods: To determine whether whole-genome sequencing (WGS) could uncover pathogenic variants missed by current molecular testing, we analysed data for 72 participants recruited to the 100,000 Genomes Project within the clinical category of SRS.

Results: In 20 participants (27% of the cohort) we identified genetic variants plausibly accounting for SRS. Coding SNV were identified in genes including *CDKN1C*, *IGF2*, *IGF1R* and *ORC1*. Maternal-effect variants were found in mothers of five participants, including two participants with imprinting disturbance and one with multi-locus imprinting disorder. Two regions of homozygosity were suggestive of UPD involving imprinted regions implicated in SRS and Temple syndrome, and three plausibly pathogenic CNVs were found, including a paternal deletion of *PLAGL1*. In 48 participants with no plausible pathogenic variant, unbiased analysis of SNVs detected a potential association with *STX4*.

Conclusion: WGS analysis can detect UPD, CNV and SNV, and is potentially a valuable addition to diagnosis of SRS and related growth-restricting disorders.

Keywords: Silver-Russell syndrome, Whole genome sequencing, multi-locus imprinting disorder, Temple syndrome

INTRODUCTION

Growth restriction is a feature of many congenital conditions, and clinical decisions are required to determine which individuals to treat and in what way. Since growth variation is continuous, not categoric, qualitative clinical criteria must be applied to make prognoses and select treatments. Moreover, pinpointing the underlying pathogenesis enables a more accurate prognosis using evidence-based medicine.

Silver-Russell syndrome (SRS) affects approximately 1 in 15,000 children and is characterised by intrauterine and postnatal growth restriction (IUGR / PNGR), along with relative macrocephaly and prominent forehead in infancy, body asymmetry, and significant feeding difficulties.[1 2] These six clinical features form the Netchine-Harbison Clinical Scoring System (NH-CSS)[3]: individuals meeting 3/6 criteria warrant referral for molecular diagnosis, whereas 4/6 features constitute a clinical diagnosis of SRS irrespective of molecular diagnosis.

In many countries a formal diagnosis of SRS is important for accessing treatment, and early, accurate molecular diagnosis optimises clinical management and genetic counselling. However, the genetic causes of SRS are numerous and account for fewer than 60% of clinically-diagnosed patients. Approximately half have loss of methylation (LOM) at Imprinting Centre 1 (IC1, also known as the *H19:IGF2* intergenic differentially methylated region or *H19:IGF2* IG-DMR) on chr11p15, or rare copy number variants (CNV) or UPD involving chr11p15.[1] 5-10% of cases show uniparental disomy (UPD) of chromosomes 7, 14, 20, 6 and 16; the (epi)genetic changes on chr14 overlap with Temple syndrome (TS) which shares clinical features with SRS.[4-6] Rare SRS cases have pathogenic short nucleotide variants (SNV) of imprinted genes on chr11p15 (*CDKN1C* and *IGF2*) or genes in the IGF2 pathway,[7-11] or have multi-locus imprinting disorder (MLID) potentially associated with trans-acting mutations altering imprinting control.[12] In cases with none of these mutations, numerous differential diagnoses may be considered.[1]

Recent years have seen increasing adoption of genomic technology in diagnosis, including gene-panel or exome testing. [13 14] There is a general expectation that whole-genome sequencing (WGS) – analysis of unbiased, uncaptured sequence from genomic DNA – will be a useful tool in the diagnosis of short stature syndromes. In the 100,000 Genomes Project (100KGP), NHS clinicians from a range of specialties worked with Genome Medicine Centres (GMCs) across the UK, to recruit families for whom standard NHS testing had not identified a cause of disease. Recruiting clinicians were responsible for choosing best-fit categories for recruited participants based on their clinical features. Initial WGS analysis focused on curated gene lists for the disorders for which participants were referred, and high-confidence pathogenic findings were returned via the GMCs. In parallel, research groups in the Genomics England Clinical Interpretation Partnership (GECIPs) studied anonymised WGS data, seeking novel genetic signatures of disease.

73 participants were recruited to 100KGP with a clinical suspicion of SRS. We analysed WGS data from these participants and their families to establish whether whole genome testing was a useful way of uncovering plausible pathogenic variants associated with clinical features of SRS.

MATERIALS AND METHODS

Participants

WGS data were available from 72 participants recruited in the SRS category, and family members. One participant had data in Hg37 and was excluded from further analysis. For the participants studied, 61 pedigrees included both parental samples, 7 had the mother's only, 1 had the father's only, and one proband had neither parental sample. In families 7, 30, 41 and 60, WGS was additionally available from siblings; in all these families some siblings had clinical features, in all cases but one, SRS. For other families, no data were available about siblings.

Participants were recruited by clinicians across UK GMCs. Participants were anonymised; DNA was not available for additional studies including imprinting analysis, data were analysed within the Genomics England Research Environment, clinical data were limited to demographics and Human Phenotype Ontology (HPO) terms, and contact with referring clinicians was mediated through the Research Environment. However, 12 participants were known to us through participation in the research study "Imprinting disorders – finding out why" (IDFOW: Southampton and South West Hampshire Research Ethics approval 07/H0502/85). IDFOW participants gave informed consent for molecular investigation into the cause of their disease.

WGS Data analysis

The data analysis strategy is summarised in Figure 1.

Putative genes and regions of interest: A panel of genes and genomic regions of interest was compiled from several sources, including PanelApp, differential diagnoses from the International Clinical Consensus, known imprinted regions associated with growth-restricting imprinting disorders, and genes associated with growth in published reports (Online supplementary material: Table S1).[1 13 15 16]

Single nucleotide variants (SNV) and Short Indels: Variants passing QC metrics (Missingness < 5%, Coverage >=15, GQ >=15, Allelic fraction ratio >= 0.25) were reanalysed using ANNOVAR v1.0 [17]. Potentially pathogenic variants were filtered as: minor allele frequency (MAF) <0.01 in GNOMAD and <0.03 in 100KGP; combined annotation-dependent depletion deleteriousness (CADD) score >15; present in the coding sequence of the canonical transcript; predicted benign by neither SIFT nor PolyPhen-2. Filtered variants from the panel of genes of interest (Online supplementary material: Table S1) were examined to exclude those likely benign in ClinVar and those not segregating with phenotype.

Copy number variation (CNV): CNVs were identified using Canvas[18] and Manta[19]. CNVs passing standard Illumina quality metrics were aggregated from participants with SRS and related growth disorders (n= 1290 participants), then selected if greater than 10kb, MAF <0.01 in study cohort, overlapping exonic regions of protein-coding genes or 'classical' imprinted regions (chr 6, 7, 11, 14, 15, 20), and not found in apparently healthy participants. Filtered CNVs where examined to exclude those common in GNOMAD-SV [20] and likely benign in DECIPHER.[21]

Regions of homozygosity (ROH): Regions of homozygosity (ROH) consistent with Uniparental isodisomy (iUPD) were detected using Illumina ROHcaller as extended runs of homozygous calls in a proband with altered allele fraction from parents and without alteration in read depth. Theoretical proportion of identity by descent (IBD) was calculated from the total number of ROHs per sample as a predictor for consanguinity.[22] Copy-neutral ROHs >1Mb that overlapped growth related imprinted loci in probands with IBD < 0.5% were reported as potential segmental iUPDs.

Analysis of H19 region: To overcome shortcomings in mapping of the IC1 region, reads spanning IC1 and IC2 (chr11:1670000-3000000, GRCh38) were extracted from WGS bam files and realigned using BWA-MEM, followed by variant calling using GATK4[23], and SV calling using Manta.[19]
Ethnicity PCA analysis: To circumvent spurious association, SRS participants with pausible genetic causes (n=20), high consanguinity (n=1) and known 11p15 LOM (n=3) were excluded. Data from the remaining 48 participants and 8204 unrelated (Kinship coefficient 0.04419417) cancer germline samples from 100KGP were subjected to PCA ancestry analysis as implemented in PC-AIR.[24] Case and control samples were prepared in PLINK file format using PLINK v1.9, and PCs and loading for the samples were calculated using 30,000 autosomal SNPs (MAF > 5%, Coverage > 99%, Inbreeding coefficient > -0.1, LD pruning R2 < 0.1, HWE p-value > 0.01) restricted to variants present in the Genomics England aggregated dataset and 1KGP dataset.
Gene-Burden test of protein coding variants: Filtered variants were collapsed per gene, and subjects with at least one variant were counted per group and tested for association with growth restriction. Fisher's exact test with Bonferroni correction for multiple testing (P value < 0.05) was used to determine genome-wide significance.
SKAT-O test of protein coding variants: The optimized Sequence Kernel Association test (SKAT-O) was applied to rare variants, to increase the power of discovery under different inheritance models by combining variance-component and burden tests. Filtered variants as specified above were analysed using the implemented SKAT-O function in SKAT R-package v2.0.1 with default parameters for binary SKAT-O.[25] The fitting model was corrected for SNP-Weight using MAF, gender, and the first five principal components of the ethnicity PCA described above. Variants were collapsed considering only the protein-coding region in canonical transcripts in GRCh38.
Genomic visualisation: Sequencing reads for SNVs & short indels, and breakpoints of deletions and duplications, were visualised for validation on Integrative Genomes Viewer (IGV).[26] For segmental UPDs and large CNVs, variant allele fraction and sequencing depth were visualised using R v3.5.1.
Methylation analysis of MLID cases: Imprinting was assessed by targeted analysis as previously described [27] or by methylation-specific multiplex ligation probe-dependent amplification assay (ME30, ME032 and ME034; MRC Holland, Amsterdam, The Netherlands).

RESULTS

Clinical and epigenetic features

Participant data included year of birth (range 1981-2017), genetic sex (39 male, 33 female), ethnicity, family members (affected or unaffected), and clinical features reported as Human Phenotype Ontology (HPO) terms. A median of 7 clinical features were reported with a range 0-17 features. 50% of participants had ≥3 features of the NH-CSS (Online supplementary material: Table S2). Some had features atypical of SRS, most notably 16 participants with reported intellectual or developmental delay. This is a feature of some subtypes of SRS but it likely reflects a selection bias towards atypical SRS in this real-world cohort.

Two parents of participants had reported phenotypes, one SRS and one intellectual disability. In four families, siblings of participants had reported phenotypes, in one case intellectual disability and in all others SRS. In two participants, GMCs reported likely pathogenic variants.

Although epigenetic aberration was not an exclusion criterion, cases with known imprinting disturbance appeared to be under-represented in recruitment, perhaps because many participating clinicians considered such cases as 'diagnosed'. Five participants had reported IC1 LOM (4/5 had ≥4 NH-CSS features); two had reported normal methylation at IC1, and in 65 participants methylation status was not stated.

Findings from WGS using a gene panel of genes and regions implicated in SRS

WGS analysis uncovered 23 potentially pathogenic genetic variants affecting 20 participants (Table 1). These variants, and additional SNV that potentially contribute to the clinical presentation of participants, are listed in Online supplementary material: Table S3.

Coding SNVs

Initial analysis focused on rare, protein-latering SNV an extensive list of genes associated with growth and growth restriction (Online supplementary material: Table S1). Three participants had variants within genes on chr11p15 directly associated with SRS (Online supplementary material: Figure S1). One had the maternally-inherited *CDKN1C* variant R281I, neighbouring the PCNA-binding region associated with variants causing IMAGe syndrome, and two had paternally inherited coding variants in *IGF2*.

Five mothers of SRS probands had heterozygous variants in *NLRP2*, *NLRP7* and *PADI6*. Among these, the two with maternal *NLRP7* variants were among those with LOM of IC1; two with maternal *NLRP2* variants had no detected methylation disturbance, and the participant with a maternal *PADI6* variant had multi-locus imprinting disorder, including LOM of the 14q32 IG-DMR, consistent with Temple syndrome (Figure 2).

Compound heterozygosity for pathogenic variants was found in two probands: one involved *ORC1*, associated with Meier-Gorlin syndrome; another involved *IGF1R* (Online supplementary material: Figures S2, S3a). One participant inherited an *IGF1R* SNV from a father also reportedly affected by short stature (Online supplementary material: Figure S3b). One participant inherited a variant in *LZTR1*, associated with Noonan syndrome; another had a variant in *PIK3R1*, associated with SHORT syndrome, though lack of parental samples prevented determination of inheritance (data not shown).

Two diagnoses were made by the 100KGP Genomic Medicine Centres (GMC). One participant had pathogenic variants in *SON* and *SCN8*; another had a pathogenic variant in *KDM6A* (data not shown). Of these only KDM6A was in our gene panel.

Structural variants

Of 30 SVs found in participants (Online supplementary material: Table S4), one was a maternally-inherited 410kb deletion involving *IGF1* (Figure 3A); another was a mosaic deletion affecting the paternally-inherited allele of chr6q24, including *PLAGL1* (Figure 3B). In a third participant, a maternally inherited deletion of chr17q24.2, including PSMD12, was potentially relevant to clinical presentation (Figure S4); other SVs were likely benign.

Regions of homozygosity

Regions of homozygosity (ROH) were identified as a proxy for UPD. Two participants showed >1% identity by descent (IBD), but their ROH did not overlap genes or regions currently associated with SRS, nor did they overlap one another. Two participants showed extensive regions of maternal homozygosity: one spanned the imprinted region around chr14q32, consistent with Temple syndrome (Figure 4); another on chromosome 7 included the imprinted gene *PEG10*, (Online supplementary material: Figure S5) but heterodisomy for the remainder of chr7 could not be assessed due to paternal DNA being unavailable.

Noncoding variants affecting IC1

Five participants had IC1 LOM. Since the standard analysis pipeline of 100KGP did not resolve the IC1 region, presumably because of its repetitive structure, the region was re-mapped, to seek cis-acting variants potentially predisposing to LOM in these participants. No rare CNV were detected within the re-annotated region. One participant had a rare paternally-inherited variant within IC1: chr11:2000298G>A (MAF0.00016), which does not overlap described CTCF- or ZFP57-binding motifs (Online supplementary material: Figure S6).

Rare variant association tests

In 48 participants with no plausible pathogenic variants identified from our gene panel, and with IBD <0.5%, aggregated filtered variants were grouped per gene. In this dataset, the aggregated effect of rare variants was assessed using Fisher's exact test, and the optimized Sequence Kernel Association test (SKAT-O) (Online supplementary material: Figure S7). SKAT-O combined variance-component and burden tests, and enabled correction for ethnicity and gender. While uncorrected burden testing identified 5 genes with p value <-0.001, but with no obvious inflation (Figure 5.A-B), after correction using SKAT-O only one gene, *STX4*, remained significant (3 of 48 participants, compared with 30 of 8204 controls, p value = 5.14e-08) (Figure 5.C-D). Two heterozygous missense variants were found in three participants: V57M, maternally inherited, in participant 6, and M159I in participants 42 and 57, in maternal and paternal inheritance respectively (Table 1, Online supplementary material: Table S3). Rare variants in *STX4* thus suggested a potentially significant association with SRS.

DISCUSSION

In a group of 72 individuals recruited to 100KGP in the category of SRS, WGS data analysis revealed potentially pathogenic genetic SNV, CNV and UPD crossing every molecular lesion currently associated with SRS, and also variants associated with disorders phenotypically very different from SRS.

The 100KGP was a pragmatic study, based on real-world sampling of patients seen within routine clinics who could benefit from whole genome sequencing. Recruiting clinicians were responsible for choosing best-fit categories for recruited participants, and hence we assume that SRS was a reasonable diagnosis in this cohort. The reported clinical features were insufficient for a provisional diagnosis of SRS in 50% of participants, but it was impossible to ascertain whether other SRS features were present but not recorded. This cohort is representative of clinical practice, where clinical data are often incomplete; but it was additionally challenged by limited epigenetic data recording and lack of contact with referring clinicians. In a routine clinical scenario, many of the discoveries reported here would be followed up by confirmatory phenotyping and testing that was not possible with the design of this study, except for participants who were also part of the imprinting study, IDFOW.

Whereas 30-60% of individuals with clinically-diagnosed SRS have LOM of IC1, [1] in this cohort only five participants were reported to have LOM. In these participants, re-mapping and analysis of the IC1 region revealed one paternally-inherited rare variant, but this did not overlap any known transcription-factor binding sites[28] and thus was not obviously pathogenic. However, two other individuals with IC1 LOM had maternally-inherited variants in *NLRP7*, a maternal-effect gene, and a third participant, whose mother harboured a variant in *PADI6,* showed MLID with epigenetic changes consistent with TS. Therefore, of five participants whose mothers had variants in maternal-effect genes, three had imprinting disturbance detected by targeted testing. We previously observed in a research cohort with MLID that over 50% of their mothers harboured rare coding variants in maternal-effect genes; several reports now associate variants in these genes with a range of reproductive outcomes including infertility, pregnancy loss and imprinting disorders.[12 29 30] This finding, in a differently-ascertained cohort, make it reasonable to consider maternal-effect variants for individuals with isolated epigenetic errors, since current epigenetic testing may underestimate the prevalence of MLID. A maternal effect variant in a family significantly alters recurrence risks and may impact long term prognosis.

Two participants had regions of maternal homozygosity ≥5Mb overlying known imprinted loci, on chromosomes 7q21 and 14q32. An inclusion criterion for 100KGP was exclusion of alternative diagnoses, including UPD. While the small ROH here might have eluded detection, imprinting analysis of chr7 and chr14 should have detected imprinting disturbance diagnostic for both of these participants. Because of the ethical structure of 100KGP, no confirmatory testing was possible for these participants; but confirmatory testing is important, particularly for the individual with ROH of 14q32, because this is consistent with a diagnosis of Temple syndrome. Temple syndrome clinically overlaps SRS in early childhood, but has important differences, including very early puberty and metabolic change, which require early diagnosis for appropriate management.[4 5] The *SGCE/PEG10* locus at 7q21 is one of at least 4 imprinted regions on chr7, and not currently the prime candidate for its association with SRS.[2 31] The apparent maternal isodisomy of 7q21 may be flanked by heterodisomic regions but without paternal WGS data this could not be proved.

Related to this, one participant had mosaic deletion of the paternal allele of the imprinted gene *PLAGL1*, which due to its low mosaicism was missed by aCGH. While paternal *PLAGL1* duplication is associated with TNDM[32], deletion has not been reported; but upd6mat is tentatively associated with prenatal and postnatal growth restriction.[33] We believe this is the first reported case of a patient with growth restriction due to deletion of *PLAGL1*, a growth pattern predicted by studies in mouse. [34]

Several genes implicated in growth restriction were affected by putatively pathogenic coding variants. Aside from genes associated with SRS, *CDKN1C* and *IGF2,* [7-10] variants affected *IGF1R*, *ORC1* (associated with Meier-Gorlin syndrome) and *LZTR1* (associated with Noonan syndrome).[35-37] These findings parallel those recently obtained through exome and gene panel approaches. [13 14] Further potentially-pathogenic variants were identified but detailed clinical follow-up was beyond the scope of this project.
GMCs made diagnoses in two participants. One had pathogenic variants in *SON* and *SCN8*: *SON* is associated with ZTTK syndrome, where poor growth is seen alongside motor and developmental delay, and both genes are outside the differential diagnosis of SRS.[38] Another participant had a pathogenic variant in *KDM6A,* associated with Kabuki syndrome, whose clinical features include prenatal and postnatal growth restriction.[39] The presence of these diagnoses in this cohort suggests either that the syndromes have broader phenotypes than currently recognised, or that the affected participants, and potentially others, did not fully clinically accord with SRS. In line with the latter suggestion, the recorded clinical features of 50% of the cohort did not meet the NH-CSS criteria for epigenetic testing.

In 48 participants, no plausible pathogenic variants were found using a focus on CNV, ROH, or coding SNV in a gene panel for growth restriction. In these participants we attempted to explore beyond a known gene panel, performing gene burden analysis to identify rare coding variants enriched in participants versus a large control dataset. This agnostic analysis identified a single significant gene, *STX4*. Syntaxins are highly conserved proteins with essential, partially overlapping roles in intracellular vesicle trafficking. Notably, STX4 is involved in insulin granule exocytosis from pancreatic beta-cells as well as insulin-stimulated glucose uptake by skeletal muscle;[40 41] these processes are potentially relevant to the pathophysiology of SRS. However, in all cases heterozygous variants were inherited from a parent with no reported features of SRS, and the presence of both maternal and paternal inheritance did not indicate imprinting of *STX4*. Further, informatic metrics assessing tolerance of genes to variants, pLI and missense Z-scores, suggests that *STX4* is tolerant of mutations (pLI and Z-score 9.66E-05, 2.014 respectively). [42] However, other key growth restriction genes also have metrics suggesting tolerance of variation (such as *IGF2*:2.53E-02, 2.308; *GH1*: 1.74E-02, -0.351; *CUL7*: 2.24E-11, 0.482), suggesting that such metrics may not be a key guide to variant interpretation in growth restriction disorders. The small size of this cohort and the unavailability of clinical follow-up impede interpretation of *STX4* variants, and further molecular studies would be required to determine whether *STX4* is involved in SRS, potentially in combination with other genetic or environmental factors. Notwithstanding the equivocal findings from our agnostic approach, we suggest that burden testing in large, well-phenotyped cohorts should be attempted, to go beyond panels of known genes and truly exploit the potential of WGS to identify novel causes of rare disease.

This study shows that WGS analysis is a potentially valuable tool for diagnosis for individuals with clinical features of SRS. It extends the range of genes to be considered, demonstrates the indespensibility of clinical phenotyping, and illustrates the challenges of incorporating WGS into routine diagnostics. More fundamentally, it shows the challenge of defining a clinical entity like SRS in a way that reflects evolving understanding of the disorder, but remains as practical and helpful as possible for diagnosis and management, for health professionals and for families.

SRS is currently defined as a clinical entity, using clinical nosology.[1] As with other growth disorders, it is defined by qualitative clinical thresholds applied to continuously-variable metrics of growth. Besides anthropometric criteria it recognises other features; but all are 'soft', all evolve over time, and none are unique. This clinical nosology was essential when SRS was defined as an entity, since its molecular aetiology was unknown. As molecular causes of SRS have been defined, individuals with a positive molecular diagnosis have increasingly defined the cohort whose features define the clinical diagnostic criteria. The molecular diagnostic rate of SRS has risen to ~60%; but the corollary of this is that SRS is now both pleiotropic – the same molecular change associated with a range of phenotypes – and heterogeneous – similar phenotypes associated with different genetic diagnoses. Concurrently, genetic diagnosis has become technologically more agnostic, since comprehensive approaches like WGS do not require a prior clinical hypothesis, and more democratic, since a wider range of health professionals may refer individuals for genetic testing. This approach, carried to an extreme, would be a genomic survey applied in the virtual absence of clinical definition; the opposite of a clinical nosology. This study arguably shows both the potential and pitfalls of such an approach, in that a group of patients collected under a relatively broad clinical definition of SRS turned out to have a broad range of genetic changes, including some scarcely related to SRS.

What is a pragmatic way forward? 'Nosologists in all fields tend to be either "lumpers" or "splitters"'. [43] 'Lumping' represents clinical nosology: grouping patients in terms of their clinical presentation plainly guides their treatment – but this may include people whose genetics are contraindicated (e.g. Growth Hormone treatment in Bloom syndrome), or exclude those that do not meet heuristic criteria (e.g. higher birth weight in Temple syndrome preventing the appropriate use of Growth Hormone in some countries). 'Splitting' represents a genetic nosology, defining patients by their molecular disorder, and this guides precision medicine to the same extent as it subdivides patients – to the point where evidence-based management may become impracticable. Both approaches have evident strengths and weaknesses in a growth restriction disorder like SRS.
A useful compromise may be reached by combining a shared clinical term – SRS – to lump individuals with similar medical needs, with a degree of genetic splitting to stratify care. Such an approach, involving a dyadic (gene:phenotype) taxonomy for genetic disorders, has recently been proposed.[44] If the taxonomy of SRS evolves, this may reflect an evolving definition of the clinical entity. Fresh discussion among clinicians, geneticists, healthcare providers and families may be warranted to reassess the clinical criteria that prompt testing, the genetic and epigenetic changes included and excluded in its definition, and whether it constitutes a syndrome, a spectrum, or an other grouping within the matrix of growth restriction conditions.

In conclusion, our study has shown that WGS has a place in diagnosing individuals with features of Silver-Russell syndrome; but harnessing its full potential will require excellent clinical characterisation,

long term follow-up, and nuanced molecular investigation including epigenetics, mosaicism, maternal effect mutations and imprinted inheritance patterns.

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Ethics approval Southampton and South West Hampshire Research Ethics Committee.

Data availability WGS data and associated clinical data are held within the 100,000 Genomes Project Research Environment. These data can be accessed by any researcher, by application to join a GeCIP domain (www.genomicsengland.co.uk/join-a-gecip-domain/) . Informatic scripts are available upon request.

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Figure 1: Data analysis strategy

The four columns of the figure illustrate the four informatic processes applied to sequence data from all 72 participants and their families. The boxes at the foot of each column summarise the plausible pathogenic variants uncovered by each process. SNV marked with an asterisk were identified by GMCs and returned to referring clinicians as the likely causative variant.

Figure 2: SNV in PADI6 present in a participant with MLID and mother.

A: pedigree information for the family. B: IGV visualisation of the relevant sequence of PADI6 in participant (P), mother (M) and father (F), showing the variant in participant and mother. C: electropherograms of targeted DNA methylation analysis in the participant. The peaks represent amplification products from maternally-derived (red bars) and paternally-derived (blue bars) sequence. The MEG3 DMR on chr14q32 is paternally methylated, and the WRB DMR on chr21q22 is maternally methylated. Compared with DNA from a normal control individual (upper traces), the participant's DNA (lower traces) is hypomethylated at both loci.

Figure 3: Plausible pathogenic CNVs detected in two participants.

A: Maternally-inherited heterozygous deletion affecting *IGF1*. Upper panel: left, pedigree information for the family; right, ideogram showing location of deletion. Centre panel: visualisation of read depth around the 411kb deletion in the participant and mother. Lower panel: IGV visualisation of breakpoints of the deletion showing discordant reads in participant and mother.

B: de novo heterozygous, mosaic deletion affecting *PLAGL1*. Upper panel: left, pedigree information for the family; right, ideogram showing location of deletion. Middle panel: visualisation of read depth ratios for the participant and both parents (log scale: 0 = diploid read depth; black dots represent reads with depth ratio ≥0; grey dots represent reads with depth ratio <0 indicating loss), Lower panel: the allele fraction (AF) visualisation from het SNPs in participant, mother and father, coloured by parental origin (blue: paternal; red: maternal). Weighted regression method was used to detect trends in AF data from paternal and maternal alleles, and demonstrate a significant reduction in the average AF of paternal allele compared to maternal allele in the participant.

Figure 4: Plausible pathogenic region of homozygosity affecting the chr14q32 imprinted region.

A: ideogram showing location of deletion. B: visualisations of allele fraction and depth ratio in proband, mother and father, for 15Mb at 14qter. The allele fraction visualisation of the participant (top panel) shows homozygosity of SNPs in a 10.4Mb region that encompasses the imprinted 14q32 region, while read depth ratio (second panel) is normal, showing no loss or gain of genetic material. C: representation of the imprinted region on 14q32, indicating genetic location of key features in Hg38. Filled blue oblong: paternally expressed coding gene, *DLK1*. Red unfilled oblong, maternally expressed non-coding RNA, *MEG3*. Blue filled circles, the germline imprinting centre *MEG3-DLK1* IG-DMR, and the somatic *MEG3* TSS DMR. D: IGV visualisation encompassing the *MEG3-DLK1* IG-DMR and *MEG3* TSS-DMR, showing homozygous SNPs in the participant co-located with heterozygous SNPs in the mother, indicating isodisomy of maternal origin with no paternal contribution.

Figure 5: Identification of putative novel genetic associations with SRS through unbiased analysis.

Panels A and B show results of gene burden testing of rare PTVs and likely deleterious missense variants; Panels C and D show results of SKAT-O testing, corrected for PCA of ancestry, gender, and up-weighting of rare variants. Panels A and C show Manhattan plots of genes in which variants are

enriched in the case cohort (48 individuals with SRS) compared with 8204 aggregated control genomes. The negative decadic logarithm of unadjusted P-values are plotted against the chromosomal location of each gene. Genes surpassing the significance threshold are named. B and D show Q-Q plots of burden and SKAT-O analysis respectively, clearly showing *STX4* as the only gene observed significantly more than expected in the SRS cohort.

Figure 1

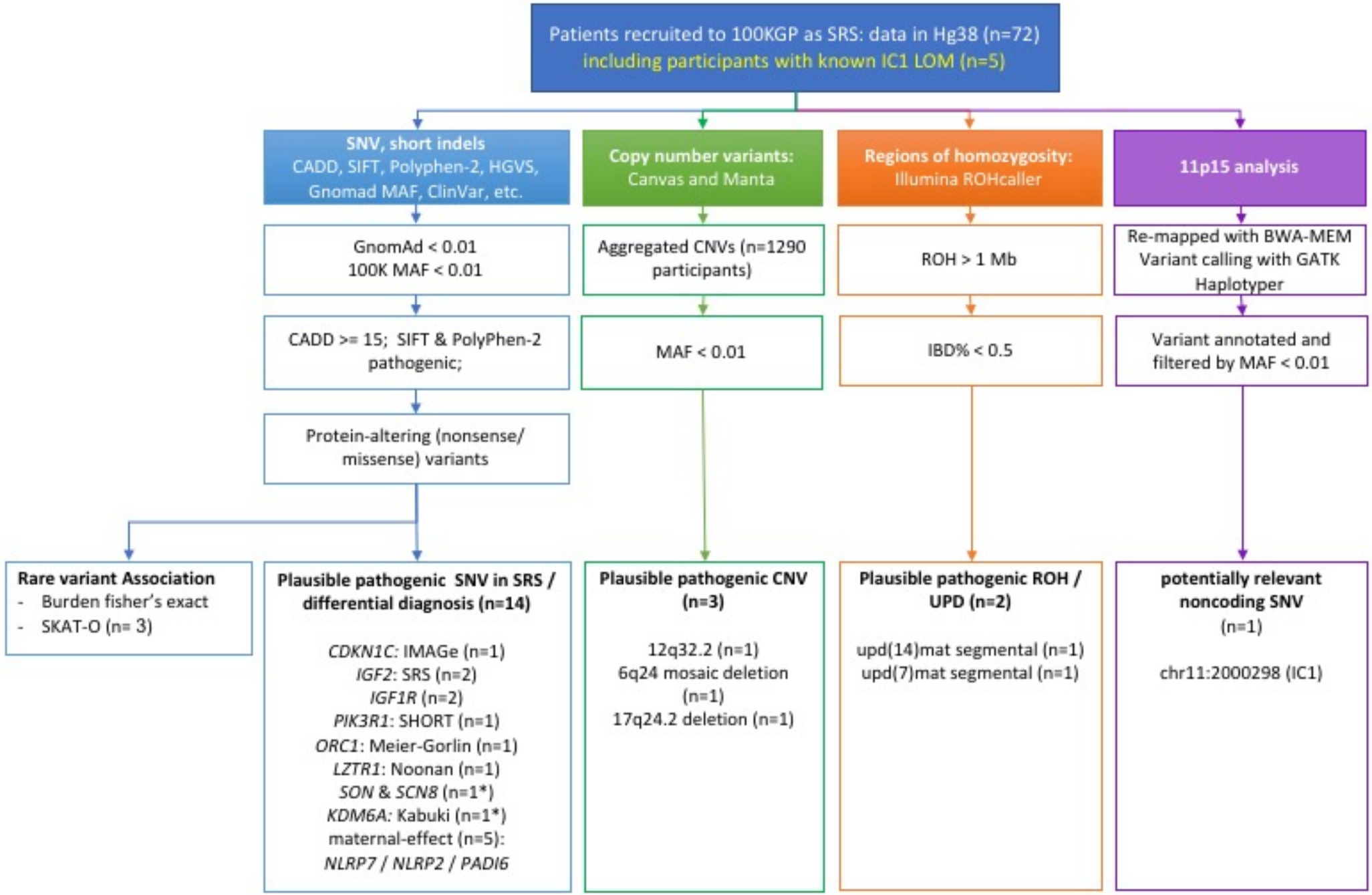
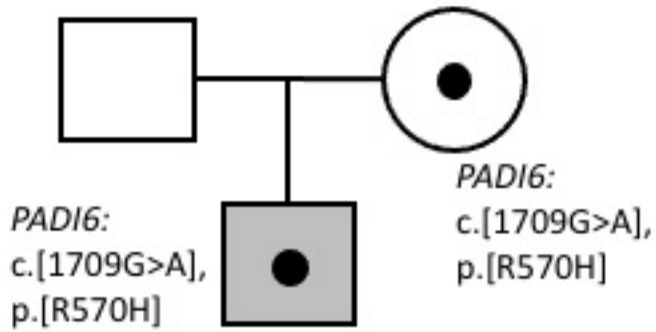
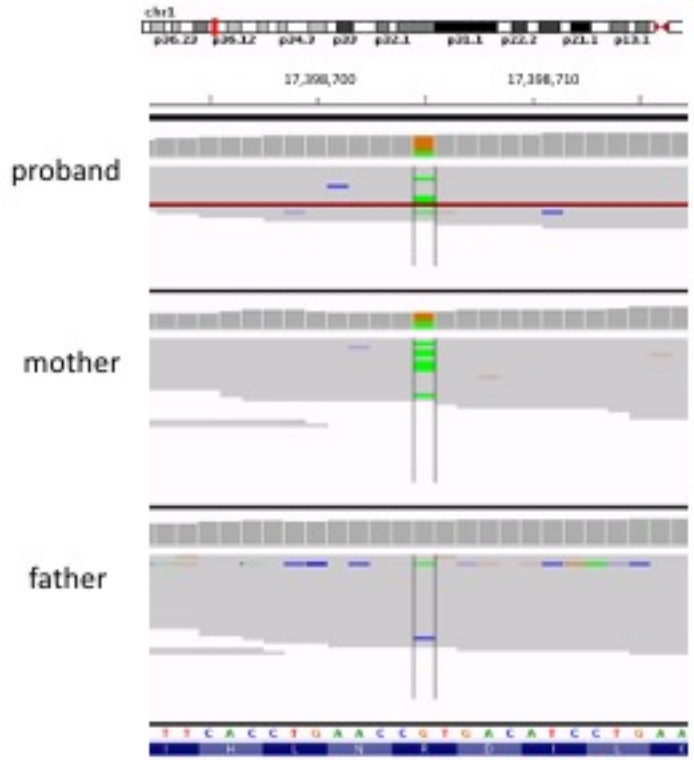

Patients recruited to 100KGP as SRS: data in Hg38 (n=72)
including participants with known IC1 LOM (n=5)
SNV, short indels
CADD, SIFT, Polyphen-2, HGVS, Gnomad MAF, ClinVar, etc.
Copy number variants:
Canvas and Manta
Regions of homozygosity:
Illumina ROHcaller
11p15 analysis
GnomAd < 0.01
100K MAF < 0.01
Aggregated CNVs (n=1290 participants)
ROH > 1 Mb
Re-mapped with BWA-MEM
Variant calling with GATK
Haplotyper
CADD >= 15; SIFT & PolyPhen-2 pathogenic;
MAF < 0.01
IBD% < 0.5
Variant annotated and filtered by MAF < 0.01
Protein-altering (nonsense/ missense) variants
Rare variant Association
- Burden fisher's exact
- SKAT-O (n= 3)
Plausible pathogenic SNV in SRS / differential diagnosis (n=14)
CDKN1C: IMAGe (n=1)
IGF2: SRS (n=2)
IGF1R (n=2)
PIK3R1: SHORT (n=1)
ORC1: Meier-Gorlin (n=1)
LZTR1: Noonan (n=1)
SON & SCN8 (n=1*)
KDM6A: Kabuki (n=1*)
maternal-effect (n=5):
NLRP7 / NLRP2 / PADI6
Plausible pathogenic CNV (n=3)
12q32.2 (n=1)
6q24 mosaic deletion (n=1)
17q24.2 deletion (n=1)
Plausible pathogenic ROH / UPD (n=2)
upd(14)mat segmental (n=1)
upd(7)mat segmental (n=1)
potentially relevant noncoding SNV
(n=1)
chr11:2000298 (IC1)

Figure 2

A



B



Participant 62 and parents

C

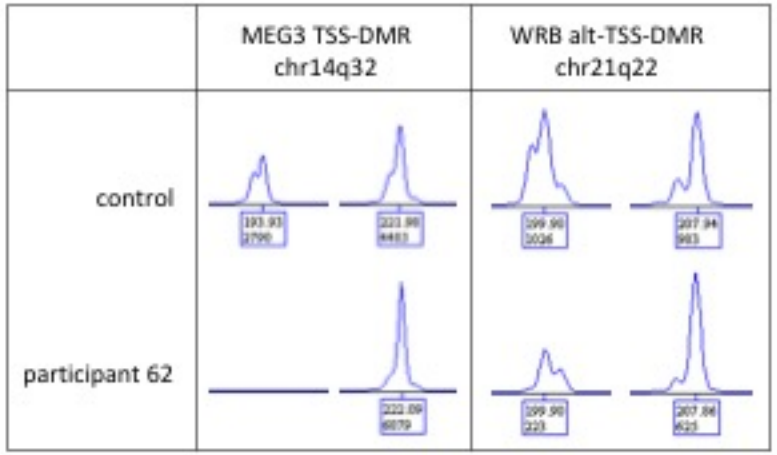
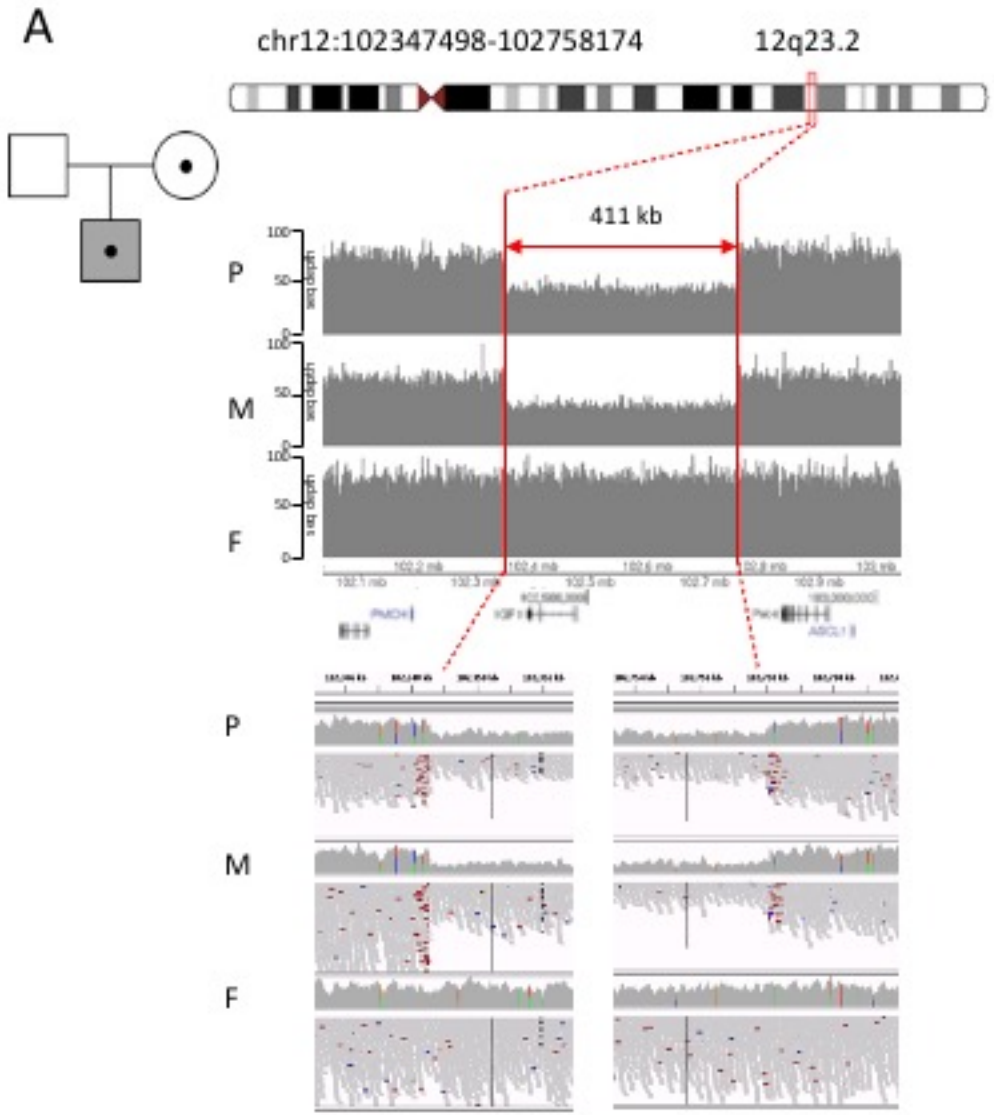
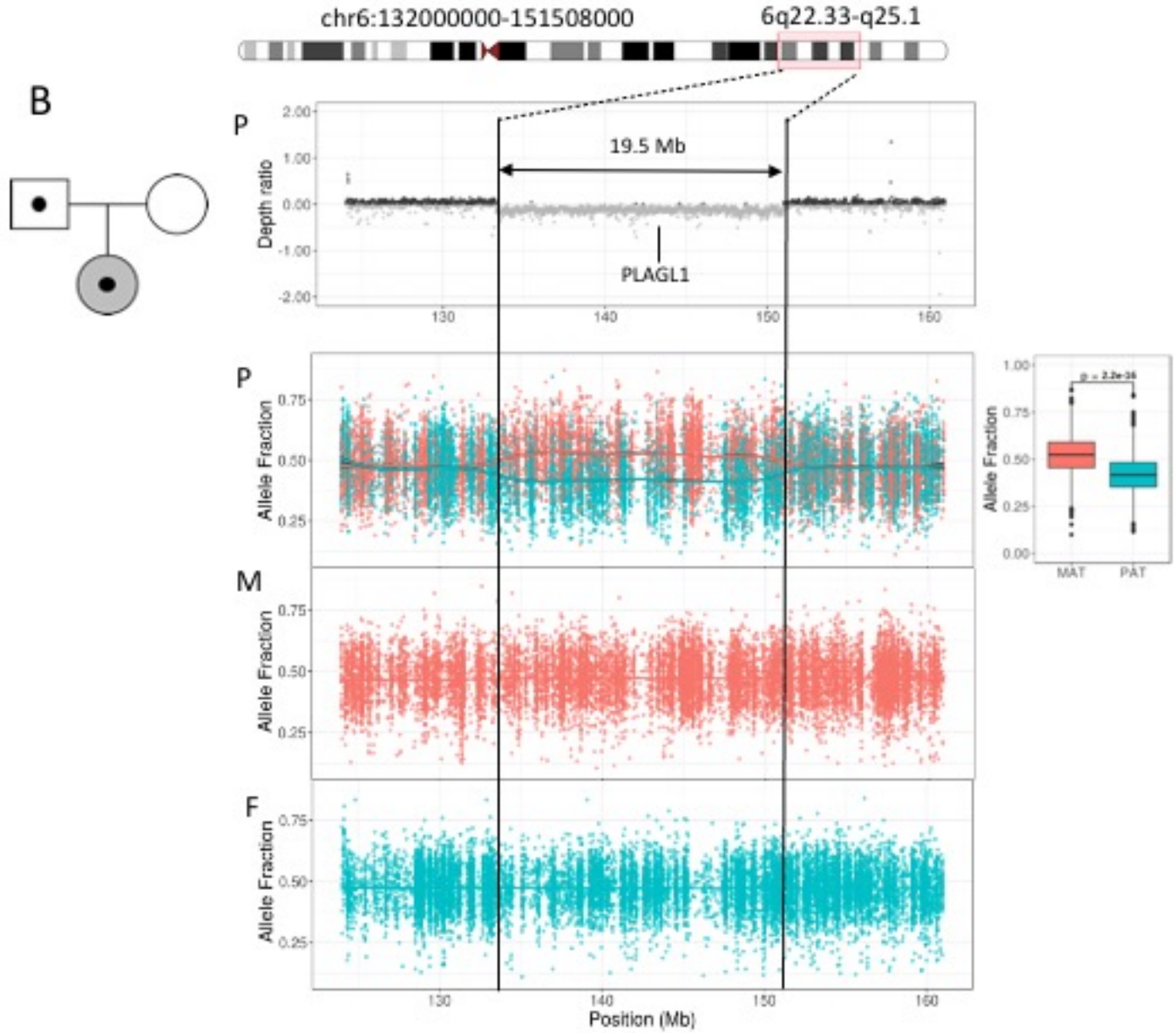


Figure 3





Het SNV (n)	AF Mean (SD)	AF PAT vs MAT (p.value)
MAT=3825 PAT = 4018	0.523 (0.100) 0.419 (0.099)	2.2e-16

Figure 4

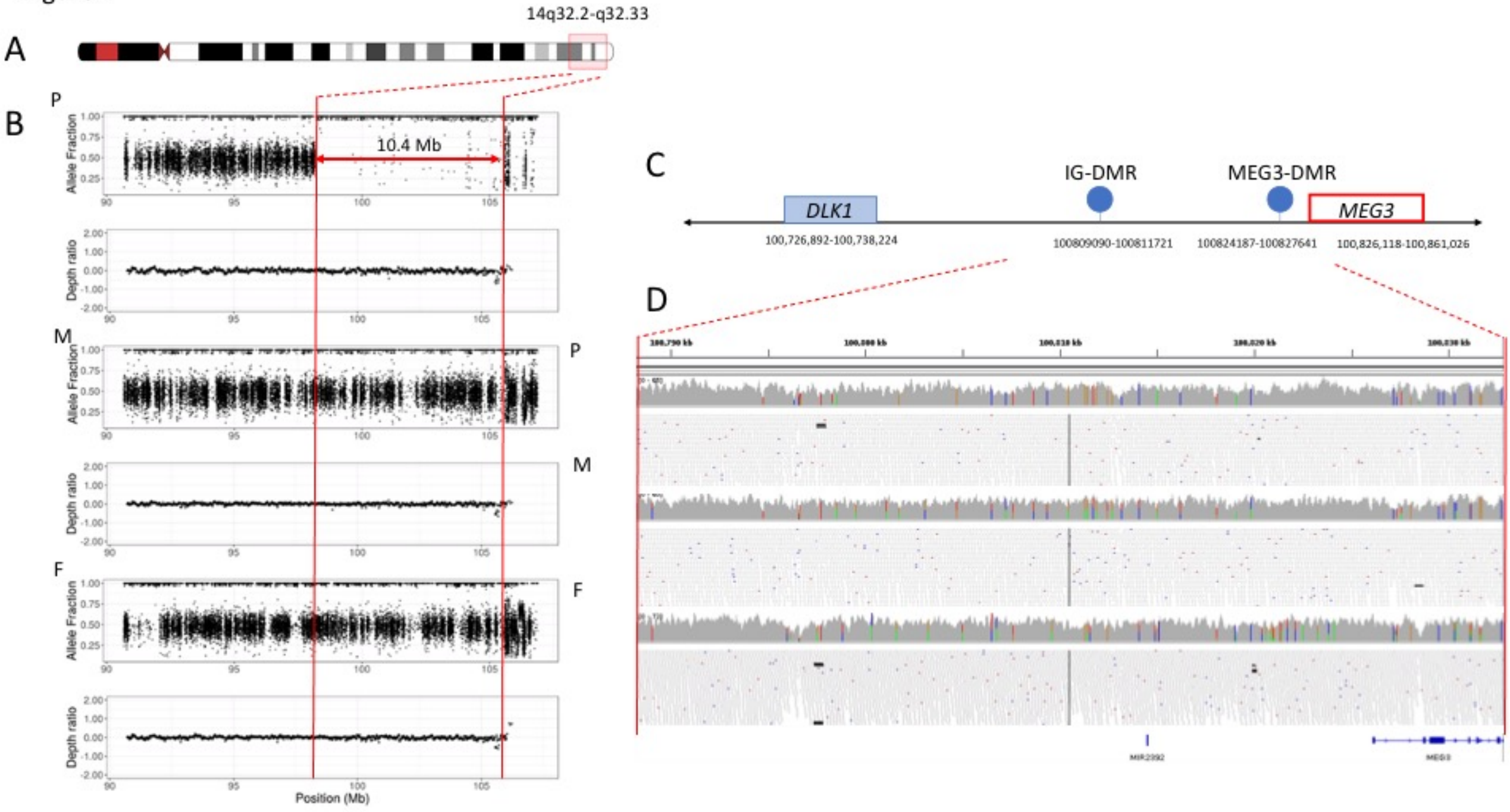
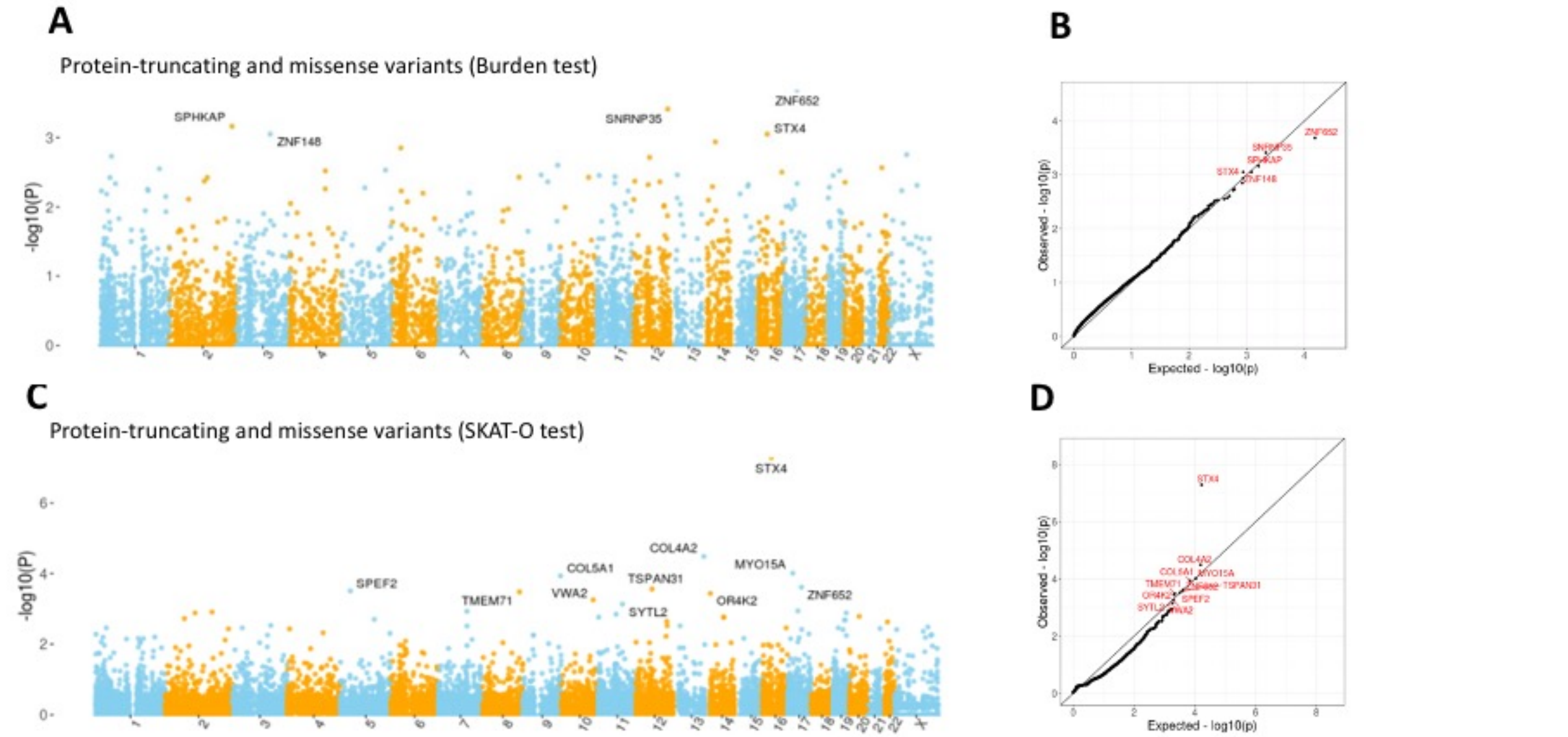

14q32.2-q32.33
A
B
P
M
F
Allele Fraction
Depth ratio
10.4 Mb
Position (Mb)
C
DLK1
IG-DMR
MEG3-DMR
MEG3
100,726,892-100,738,224
100809090-100811721
100824187-100827641
100,826,118-100,861,026
D

Figure 5


A
Protein-truncating and missense variants (Burden test)
-log10(P)
SPHKAP
ZNF148
SNRNP35
ZNF652
STX4
B
Observed - log10(p)
Expected - log10(p)
C
Protein-truncating and missense variants (SKAT-O test)
-log10(P)
SPEF2
TMEM71
VWA2
COL5A1
COL4A2
TSPAN31
SYTL2
OR4K2
MYO15A
ZNF652
STX4
D
Observed - log10(p)
Expected - log10(p)

Whole genome analysis as a tool for diagnosis in Silver-Russell syndrome.

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Supplementary Information

Supplementary Table1. Lists of genes and regions used for SNV analysis.
Supplementary Table 2A: number of NH-CSS features given for participants in the 100KGP SRS cohort.
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Supplementary Figure 1: SNVs in *CDKN1C* and *IGF2* in participants with SRS.
A: pedigree information for families of participants 18, 53 and 64. Grey filled symbols indicate participant affected by SRS; black dot indicates individual carrying the variant specified. B: ideogram of chr11; the red bar to the left of the ideogram marks chr11p15.5, where *CDKN1C* and *IGF2* are located. C: IGV visualisation of the relevant sequences in participant, mother and father, showing the variant in the affected participant and unaffected parent.

Supplementary Figure 2: Compound heterozygosity for SNVs in *ORC1* in Participant 66.
A: pedigree information for participant 66. B: ideogram of chr1; the red bar marks location of *ORC1*. C: IGV visualisation of the relevant sequences in participant, mother and father, showing both variants in the affected participant and one in each unaffected parent.

Supplementary Figure 3: SNVs in *IGF1R* in Participants 22 and 31.
A: pedigree information for participant 22; B: pedigree information for participant 31. C: ideogram of chr15; the red bar marks location of *IGF1R*. D: IGV visualisation of the relevant sequences in participant 22, mother and father, showing both variants in the affected participant and one in each unaffected parent. E: IGV visualisation showing the SNV in participant 31 and the affected father.

Supplementary Figure 4: Deletion in chr17q24 in Participant 61.
A: pedigree information for family 61. B: Ideogram of chromosome 17; red bar marks chr17q24. C: visualisation of read depth around the 185kb deletion in the participant and the mother affected by intellectual disability. D: IGV visualisation showing the breakpoints of the deletion in participant and mother.

Supplementary Figure 5: region of homozygosity affecting 7q21 in participant 25.
A: Ideogram of chromosome 7; red oblong shows region of 7q21 with homozygosity. B: visualisations of allele fraction and depth ratio in proband and mother, spanning 45Mb in chr7q. The allele fraction visualisation of the participant (top panel) shows homozygosity of SNPs in a 16.6Mb region that encompasses the imprinted PEG10/SGCE locus region, while read depth ratio (second panel) is normal, showing no loss or gain of genetic material. C: IGV visualisation encompassing the PEG10 TSS-DMR, showing homozygous SNPs in the participant co-located with heterozygous SNPs in the mother, indicating isodisomy of maternal origin with no paternal contribution.

Supplementary Figure 6: paternally-inherited variant within IC1 in participant 34.
A: pedigree information for participant 34. B: Ideogram of chromosome 11; red bar marks chr11p15. C: IGV visualisation of the relevant sequences in participant, mother and father, showing paternally-inherited variant within IC1: chr11:2000298G>A; note that the maternally-inherited variant is rs148619931, MAF 0.006. D: Sanger traces (reverse-complement strand) in participant 34 and unrelated normal control (DNA from participant 34's parents was unavailable).

Supplementary Figure 7: Illustration for sample stratification and association tests. (A) Flow chart for burden and SKAT-O tests. (B) Flow chart for Ethnicity PCA analysis. (C) visitation of first two principal components of stratified samples of 48 SRS cases (coloured blue), 8204 control genomes (coloured in red). (D) Scree plot of the first ten PCs from the PCA ancestry analysis. Frist five PCs used as covariates for SKAT-O association test.

Supplementary Text 1: the Genomics England Research Consortium

Supplementary Table1. Lists of genes and regions used for SNV analysis.

Gene	Chr	Mode of inheritance	Disorder or clinical association
LHX4	1	BIALLELIC, AD/AR, not imprinted	Combined GH deficiency
NRAS	1	BIALLELIC, AD, not imprinted	Noonan syndrome
PADI6	1	BIALLELIC, MEG, not imprinted	Multi-locus imprinting disorder
TBX19	1	BIALLELIC, AR, not imprinted	Isolated adrenocortical deficiency; Congenital adrenal hypoplasia; GH deficiency
TSHB	1	BIALLELIC, AR, not imprinted	Isolated TSH deficiency; Congenital hypothyroidism; Intellectual disability
PAPPA2	1	BIALLELIC, AR, not imprinted	IUGR and IGF abnormalities
ORC1	1	BIALLELIC, AR, not imprinted	Meier-Gorlin Syndrome
GPR161	1	BIALLELIC, AR, not imprinted	Pituitary Stalk Interruption Syndrome
TCF7L1	2	BIALLELIC, AD, not imprinted	Combined GH deficiency
SIX3	2	BIALLELIC, AD, not imprinted	Holoprosencephaly
GLI2	2	BIALLELIC, AD, not imprinted	Isolated adrenocortical deficiency; holoprosencephaly
SOS1	2	BIALLELIC, AD, not imprinted	Noonan syndrome
OBSL1	2	BIALLELIC, AR, not imprinted	3-M syndrome
IFT172	2	BIALLELIC, AR, not imprinted	GH deficiency
POMC	2	BIALLELIC, AR, not imprinted	Isolated adrenocortical deficiency; Congenital adrenal hypoplasia
ORC4	2	BIALLELIC, AR, not imprinted	Meier-Gorlin Syndrome
IHH	2	BIALLELIC, AR, not imprinted	Skeletal dysplasia
HDAC4	2	BIALLELIC, NK, not imprinted	Skeletal dysplasia;Intellectual disability
ZDBF2	2	imprinted, pat allele expressed	Nasopalpebral Lipoma-Coloboma Syndrome
POU1F1	3	BIALLELIC, AD/AR, not imprinted	GH deficiency
HESX1	3	BIALLELIC, AD/AR, not imprinted	Isolated GH deficiency; combined GH deficiency
SOX2	3	BIALLELIC, AD, not imprinted	Hypogonadotropic hypogonadism
PROK2	3	BIALLELIC, AD, not imprinted	Kallmann Syndrome; Hypogonadotropic hypogonadism
RAF1	3	BIALLELIC, AD, not imprinted	Noonan syndrome
RASA2	3	BIALLELIC, AD, not imprinted	Noonan syndrome
ROBO1	3	BIALLELIC, AD, not imprinted	Pituitary Stalk Interruption Syndrome
FANCD2	3	BIALLELIC, AR, not imprinted	Fanconi Anemia; Congenital Anomalies
MRAS	3	BIALLELIC, AD, not imprinted	Noonan syndrome
PITX2	4	BIALLELIC, AD, not imprinted	GH deficiency; Glaucoma (developmental)
FGFR3	4	BIALLELIC, AD, not imprinted	Hypogonadotropic hypogonadism
GNRHR	4	BIALLELIC, AR, not imprinted	GH deficiency; Hypogonadotropic hypogonadism
PCSK1	5	BIALLELIC, AD/AR, not imprinted	Isolated adrenocortical deficiency; TSH deficiency
PIK3R1	5	BIALLELIC, AD, not imprinted	SHORT syndrome
PROP1	5	BIALLELIC, AR, not imprinted	GH deficiency
GHR	5	BIALLELIC, AR, not imprinted	Isolated GH deficiency
RHOBTB3	5	imprinted, pat allele expressed	placental imprinting
KHDC3L	6	BIALLELIC, MEG, not imprinted	Multi-locus imprinting disorder
CUL7	6	BIALLELIC, AR, not imprinted	3-M syndrome
ZFP57	6	BIALLELIC, AR, not imprinted	Transient neonatal diabetes
PLAGL1	6	imprinted, pat allele expressed	Transient neonatal diabetes
CRYBG1	6	imprinted, pat allele expressed	placental imprinting
GCK	7	BIALLELIC, AD/AR, not imprinted	Diabetes - neonatal onset; Congenital hyperinsulinism

GHRHR	7	BIALLELIC, AD/AR, not imprinted	Isolated GH deficiency
DLX5	7	BIALLELIC, AD/AR, not imprinted	Skeletal dysplasia
GLI3	7	BIALLELIC, AD, not imprinted	GH deficiency, hypopituitarism
SHH	7	BIALLELIC, AD, not imprinted	Holoprosencephaly
BRAF	7	BIALLELIC, AD, not imprinted	Noonan syndrome
CPA1	7	BIALLELIC, NK, not imprinted	SRS - potential candidate
CPA4	7	BIALLELIC, NK, not imprinted	SRS - potential candidate
DDC	7	BIALLELIC, NK, not imprinted	Not imprinted, chr7p12
FOXP2	7	not imprinted, NK	Not Imprinted chr7q; Intellectual disability
IGFBP1	7	BIALLELIC, NK, not imprinted	SRS - potential candidate
IGFBP3	7	BIALLELIC, NK, not imprinted	SRS - potential candidate
CPA5	7	BIALLELIC, NK, not imprinted	Not imprinted, chr7q32
COPG2	7	not imprinted, NK	Not imprinted, chr7q32
COPG2IT1	7	not imprinted, NK	Not imprinted, chr7q32
GRB10	7	imprinted, mat allele expressed	SRS candidate gene
PHKG1	7	imprinted, mat allele expressed	Neuromuscular disorders; Undiagnosed metabolic disorders
KLF14	7	imprinted, mat allele expressed	placental imprinting
SGCE	7	imprinted, pat allele expressed	myoclonic dystonia
PEG10	7	imprinted, pat allele expressed	myoclonic dystonia
KLHDC10	7	imprinted, pat allele expressed	placental imprinting
MESTIT	7	imprinted, pat allele expressed	SRS candidate gene
MEST	7	imprinted, pat allele expressed	SRS candidate gene
FGFR1	8	BIALLELIC, AD, not imprinted	Hypogonadotropic hypogonadism; GH deficiency; Hydrocephalus
TRHR	8	BIALLELIC, AR, not imprinted	Isolated TSH deficiency; Congenital hypothyroidism
NBN	8	BIALLELIC, AR, not imprinted	Nijmegen syndrome
PLAG1	8	BIALLELIC, AD, not imprinted	SRS
ZFAT	8	imprinted, pat allele expressed	placental imprinting
NPR2	9	BIALLELIC, AD/AR, not imprinted	Skeletal dysplasia
PTCH1	9	BIALLELIC, AD, not imprinted	Holoprosencephaly
LHX3	9	BIALLELIC, AR, not imprinted	Combined GH deficiency
FANCC	9	BIALLELIC, AR, not imprinted	Fanconi Anemia; Congenital Anomalies
FANCG	9	BIALLELIC, AR, not imprinted	Fanconi Anemia; Congenital Anomalies
ROR2	9	BIALLELIC, AR, not imprinted	Robinow syndrome; Skeletal dysplasia;Limb disorders; Clefting
FGF8	10	BIALLELIC, AD, not imprinted	Hypogonadotropic hypogonadism
SHOC2	10	BIALLELIC, AD, not imprinted	Noonan syndrome
CDON	11	BIALLELIC, AD, not imprinted	GH deficiency; Holoprosencephaly; Intellectual disability
CBL	11	BIALLELIC, AD, not imprinted	Noonan syndrome
RRAS2	11	BIALLELIC, NK, not imprinted	Noonan syndrome
HRAS	11	BIALLELIC, AD, not imprinted	Noonan syndrome
RIT1	11	BIALLELIC, AD, not imprinted	Noonan syndrome
PHLDA2	11	imprinted, mat allele expressed	growth restriction
CDKN1C	11	imprinted, mat allele expressed	IMAGe syndrome
H19	11	imprinted, mat allele expressed	SRS/Beckwith-Wiedemann syndrome
KCNQ1	11	imprinted, mat allele expressed	SRS/Beckwith-Wiedemann syndrome
KCNQ1OT1	11	imprinted, mat allele expressed	SRS/Beckwith-Wiedemann syndrome

IGF2	11	imprinted, pat allele expressed	SRS/Beckwith-Wiedemann syndrome
TRPV4	12	BIALLELIC, AD, not imprinted	Neuromuscular disorders; Skeletal dysplasia
A2ML1	12	BIALLELIC, AD, not imprinted	Noonan syndrome
HMGA2	12	BIALLELIC, AD, not imprinted	SRS
KRAS	12	BIALLELIC, AD, not imprinted	Noonan syndrome
PTPN11	12	BIALLELIC, AD, not imprinted	Noonan syndrome
IGF1	12	BIALLELIC, AR, not imprinted	SRS; Growth failure in early childhood
ZIC2	13	BIALLELIC, AD, not imprinted	Holoprosencephaly
OTX2	14	BIALLELIC, AD, not imprinted	Isolated GH deficiency; combined GH deficiency
SOS2	14	BIALLELIC, AD, not imprinted	Noonan syndrome
YY1	14	BIALLELIC, AD, not imprinted	Skeletal dysplasia; Early onset dystonia;Intellectual disability
MEG3	14	imprinted, mat allele expressed	Temple Syndrome
RTL1	14	imprinted, pat allele expressed	Temple syndrome
DLK1	14	imprinted, pat allele expressed	Temple syndrome
IGF1R	15	BIALLELIC, AD/AR, not imprinted	SRS; Growth failure in early childhood
MAP2K1	15	BIALLELIC, AD, not imprinted	Noonan syndrome
BLM	15	BIALLELIC, AR, not imprinted	Bloom syndrome
ARNT2	15	BIALLELIC, AR, not imprinted	Combined GH deficiency
CEP152	15	BIALLELIC, AR, not imprinted	Seckel syndrome
ACAN	15	BIALLELIC, NK, not imprinted	Sponyloepimetaphseal dysplasia - Extracellular matrix regulation
SRCAP	16	BIALLELIC, AD/AR, not imprinted	Floating Harbour syndrome
ANKRD11	16	BIALLELIC, AD, not imprinted	KBG Syndrome
CREBBP	16	BIALLELIC, AD, not imprinted	Rubinstein-Taybi syndrome; Skeletal dysplasia
FANCA	16	BIALLELIC, AR, not imprinted	Fanconi Anemia; Congenital Anomalies
IGFALS	16	BIALLELIC, AR, not imprinted	IUGR and IGF abnormalities
CDT1	16	BIALLELIC, AR, not imprinted	Meier-Gorlin Syndrome
ORC6	16	BIALLELIC, AR, not imprinted	Meier-Gorlin Syndrome
CTCF	16	BIALLELIC, NK, not imprinted	Fetal anomalies; Intellectual disability;Clefting
ZNF597	16	imprinted, mat allele expressed	Prenatal growth retardation and dysmorphic features
GH1	17	BIALLELIC, AD/AR, not imprinted	Isolated growth hormone deficiency
KANSL1	17	BIALLELIC, AD, not imprinted	Koolen-de Vries syndrome;17q21.31 microdeletion syndrome
NF1	17	BIALLELIC, AD, not imprinted	Neurofibromatosis-Noonan Syndrome
COL1A1	17	BIALLELIC, AD, not imprinted	Skeletal dysplasia; Growth failure in early childhood
RNPC3	17	BIALLELIC, AR, not imprinted	Isolated growth hormone deficiency
STAT5B	17	BIALLELIC, AR, not imprinted	IUGR and IGF abnormalities
CDC6	17	BIALLELIC, AR, not imprinted	Meier-Gorlin Syndrome
TRIM37	17	BIALLELIC, AR, not imprinted	Mulibrey Nanism; Growth failure in early childhood; Undiagnosed metabolic disorders
TGIF1	18	BIALLELIC, AD, not imprinted	Holoprosencephaly
RAX	18	BIALLELIC, AR, not imprinted	Combined Growth hormone deficiency
MC2R	18	BIALLELIC, AR, not imprinted	Isolated adrenocortical deficiency; Congenital adrenal hypoplasia
RRAS	19	BIALLELIC, NK, not imprinted	Noonan syndrome
MAP2K2	19	BIALLELIC, AD, not imprinted	Noonan syndrome
DNMT1	19	BIALLELIC, AD, not imprinted	SRS - potential candidate
NLRP2	19	BIALLELIC, MEG, not imprinted	Multi-locus imprinting disorder

NLRP5	19	BIALLELIC, MEG, not imprinted	Multi-locus imprinting disorder
NLRP7	19	BIALLELIC, MEG, not imprinted	Multi-locus imprinting disorder
CCDC8	19	BIALLELIC, AR, not imprinted	3-M syndrome
PNPLA6	19	BIALLELIC, AR, not imprinted	Oliver–McFarlane and Laurence–Moon syndrome; GH and gonadotrophin deficiencies
ZNF331	19	imprinted, pat allele expressed	growth restriction
FOXA2	20	BIALLELIC, AD, not imprinted	Combined GH deficiency; congenital hyperinsulinism;childhood-onset diabetes
GHRH	20	BIALLELIC, AR, not imprinted	GH deficiency
PROKR2	20	BIALLELIC, AR, not imprinted	Kallmann Syndrome; GH deficiency
KCNE1	21	BIALLELIC, AR, not imprinted	Long QT syndrome
PCNT	21	BIALLELIC, AR, not imprinted	MOPDII
LZTR1	22	BIALLELIC, AD, not imprinted	Noonan syndrome
SOX3	X	X-linked, XLR	Combined GH deficiency
BTK	X	X-linked, XLR	GH deficiency
EIF2S3	X	X-linked, XLR	GH deficiency; TSH deficiency; Diabetes - neonatal onset; Clefting
KAL1	X	X-linked, XLR	Hypogonadotropic hypogonadism
TBL1X	X	X-linked, XLR	Isolated TSH deficiency; Congenital hypothyroidism
IGSF1	X	X-linked, XLR	Isolated TSH deficiency; GH deficiency; Congenital hypothyroidism
KDM6A	X	X-linked, XLR	Kabuki syndrome; Congenital hyperinsulinism
SHOX	X	X-linked, XLR	Skeletal dysplasia;Limb disorders

AD: autosomal dominant; AR: autosomal recessive; Chr:chromosome; GH: Growth Hormone; IGF: insulin-like growth factor; IUGR:intrauterine growth restriction; MEG: maternal-effect gene; NK: not known; TSH: thyroid-stimulating hormone; XLR: X-linked recessive

Supplementary Table 2

Table 2A: number of NH-CSS features given for participants in the 100KGP SRS cohort.

NH-CSS features	Number of participants	Number of participants with IC1 LOM
0	3	0
1	15	0
2	18	0
3	9	1
4	17	1
5	10	3
6	0	0
≤2	36 (50%)	
≥3/6	36 (50%)	1
≥4	27 (37.5%)	4
≥4*	12 (16.7%)	2

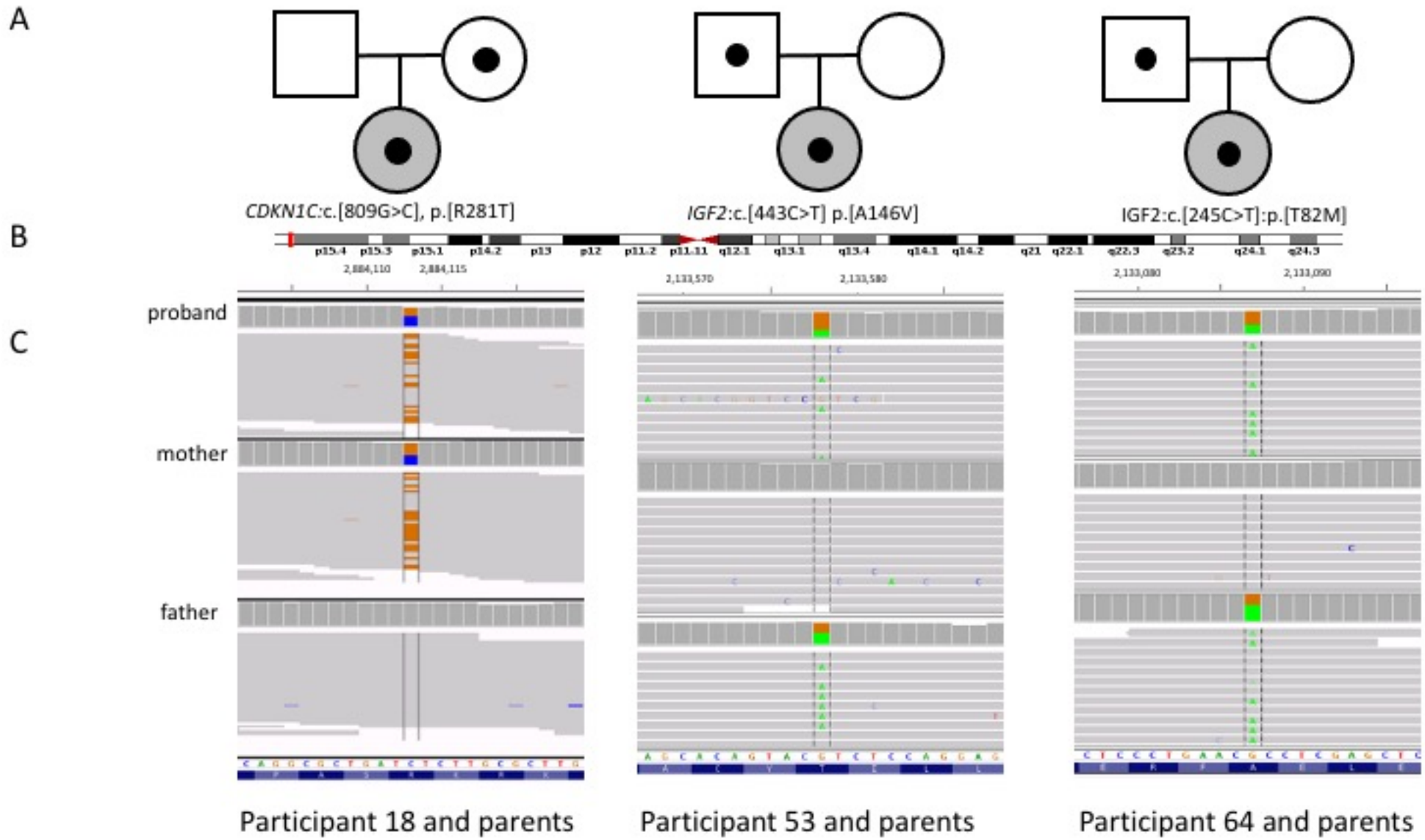
*meeting full definition of clinical SRS: ≥4/6 NH-CSS criteria, including prominent forehead and relative macrocephaly

Table 2B: features of the Netchine-Harbison Clinical Scoring System (NH-CSS)

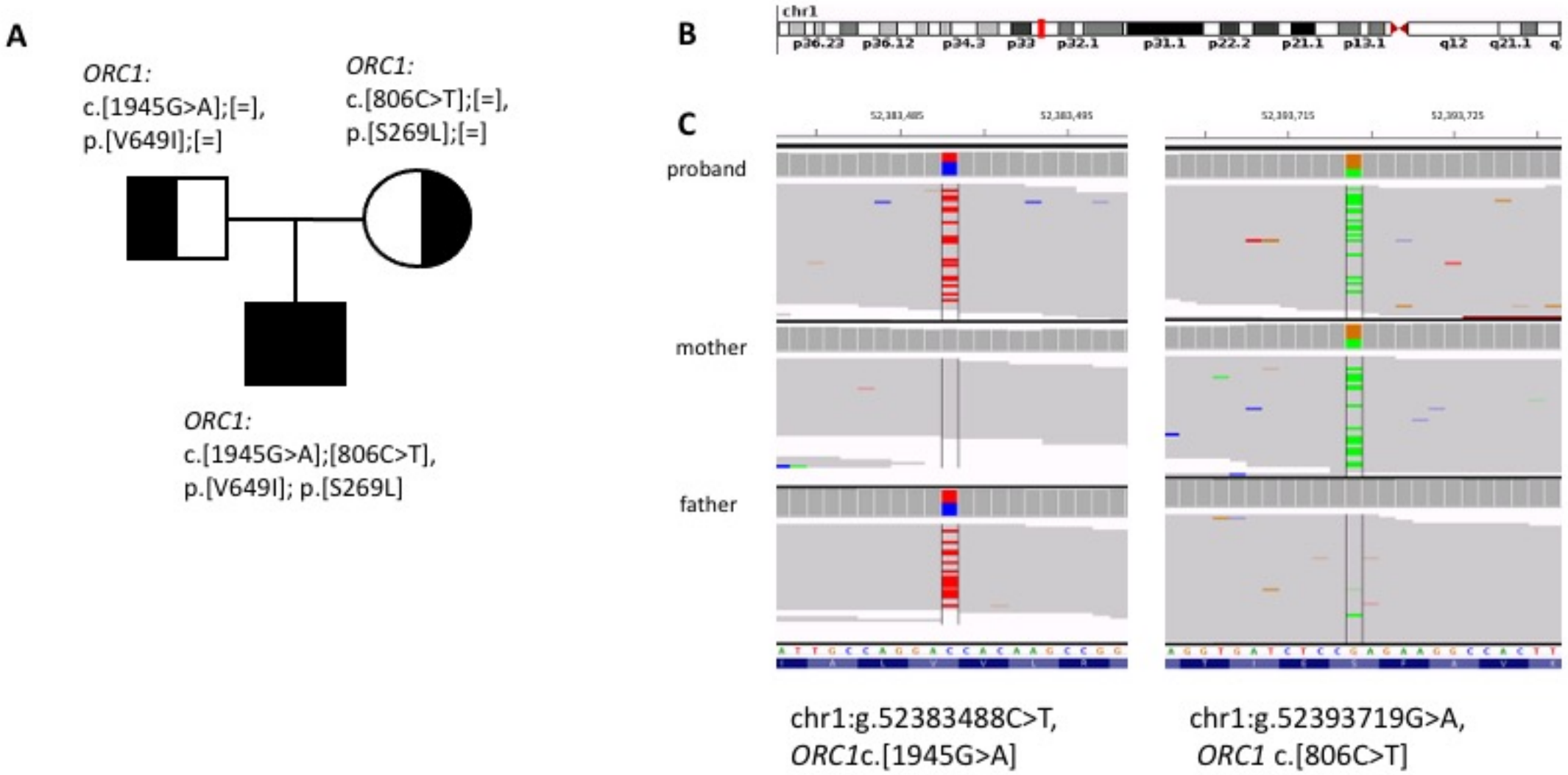
Clinical criterion	Definition
SGA	(birth weight and/or birth length) ≤−2 SDS for gestational age
Postnatal growth failure	Height at 24 ± 1 months ≤−2 SDS or height ≤−2 SDS below mid-parental target height
Relative macrocephaly at birth	Head circumference at birth ≥1.5 SDS above birth weight and/or length SDS
Protruding forehead	Forehead projecting beyond the facial plane on a side view as a toddler (1–3 years)
Body asymmetry	LLD of ≥0.5 cm or arm asymmetry or LLD <0.5 cm with at least two other asymmetrical body parts (one non-face)
Feeding difficulties and/or low BMI	BMI ≤−2 SDS at 24 months or current use of a feeding tube or cyproheptadine for appetite stimulation

Taken from Wakeling et al, 2017. Individuals scoring three or more warrant molecular testing for SRS. A clinical diagnosis of SRS is considered if a patient scores at least four of six from these criteria. If all molecular tests are normal and differential diagnoses have been ruled out, patients scoring at least four of six criteria, including both prominent forehead and relative macrocephaly, should be diagnosed as clinical SRS. LLD, leg length discrepancy; SDS, SD score; SGA, small for gestational age.

Supplementary Figure 1

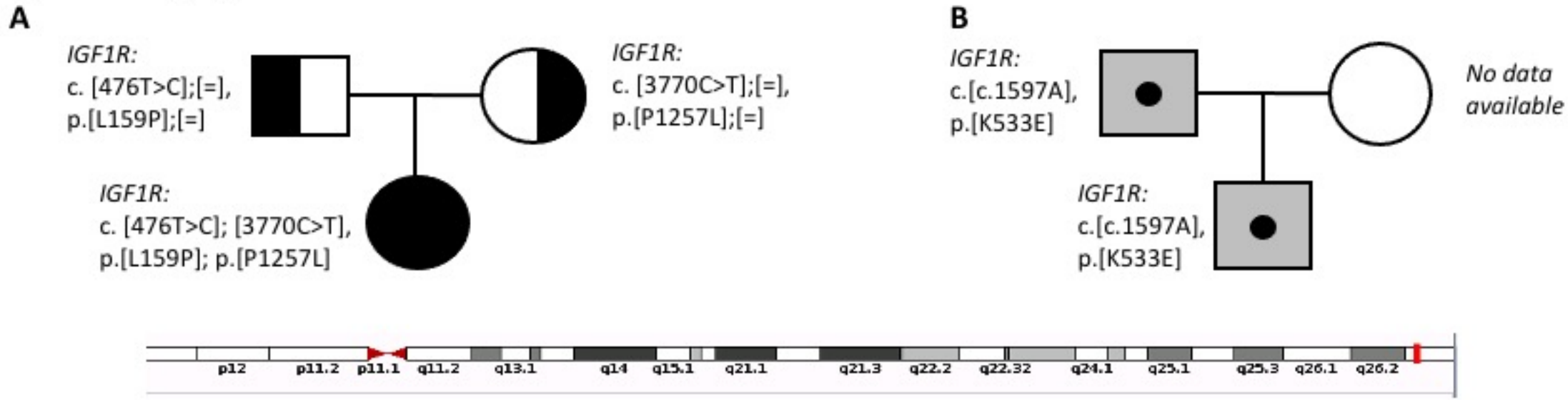


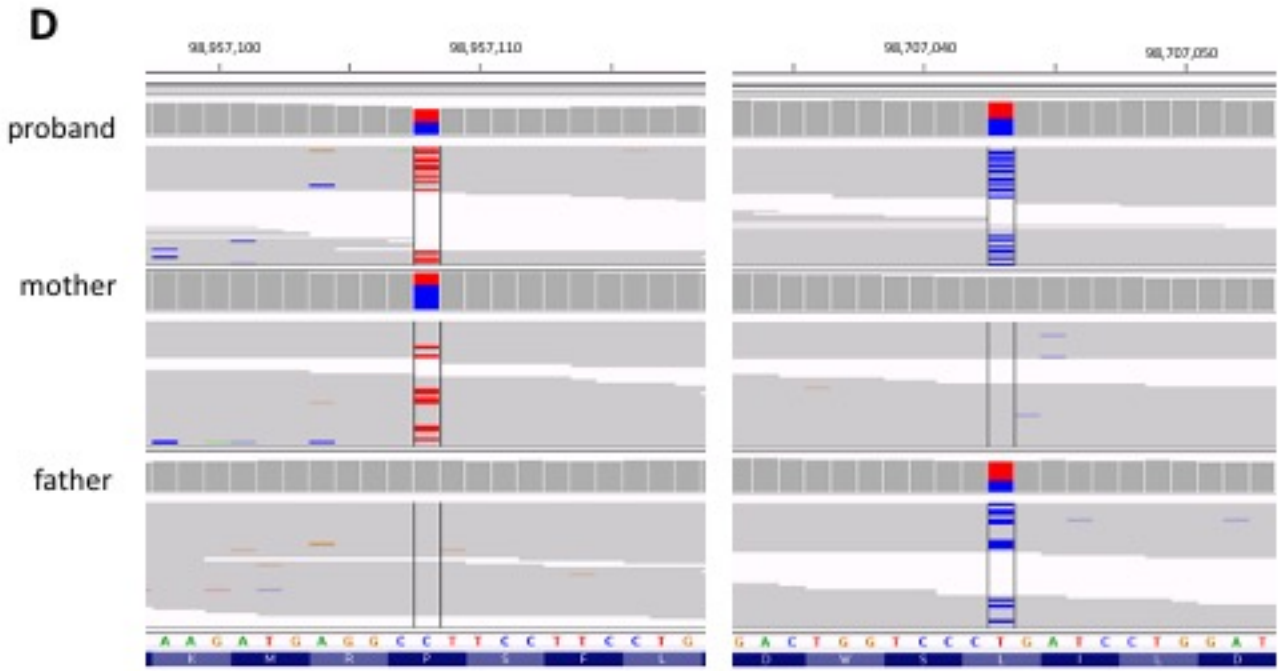
Supplementary Figure 2



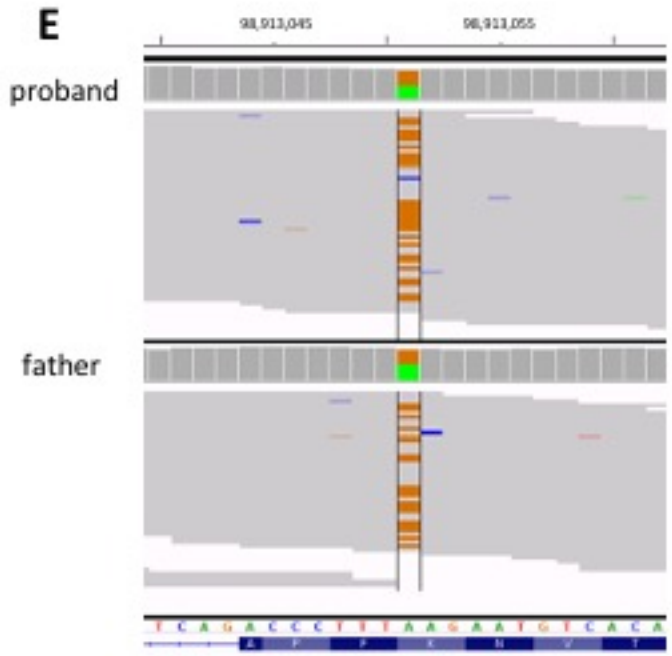
Participant 66 and parents

Supplementary Figure 3





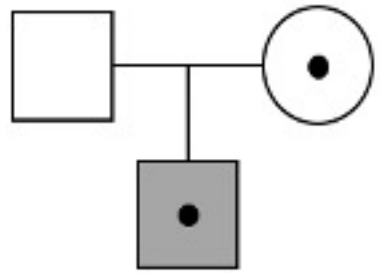
Participant 22 and parents



Participant 31 and father

Supplementary Figure 4

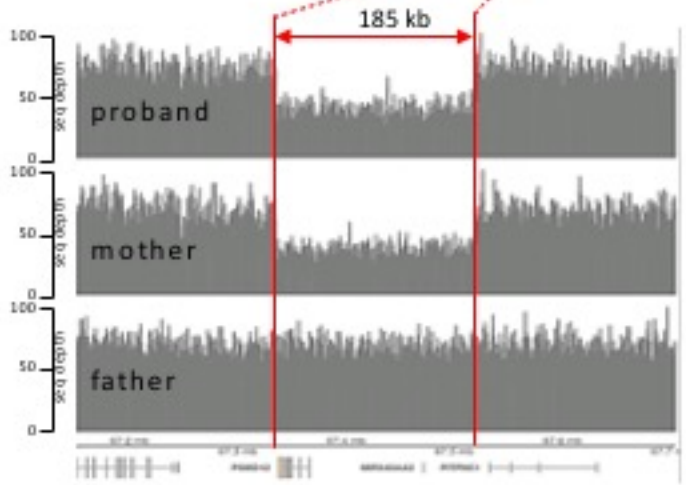
A



Participant 61 and parents

B

C



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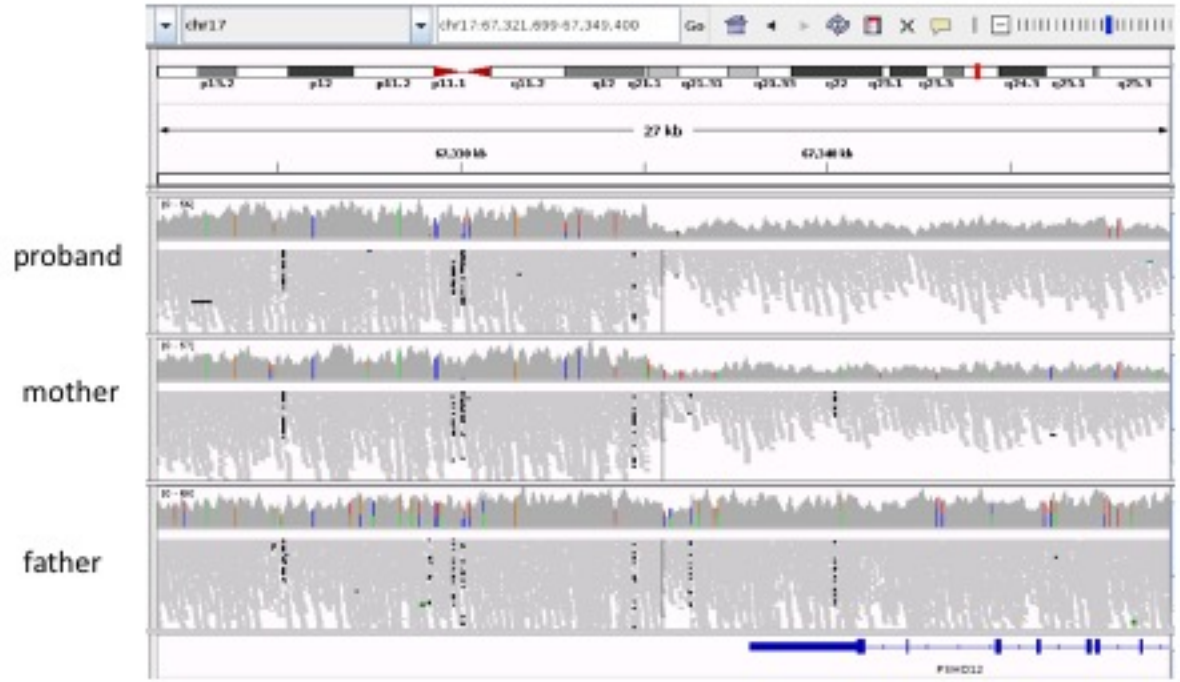
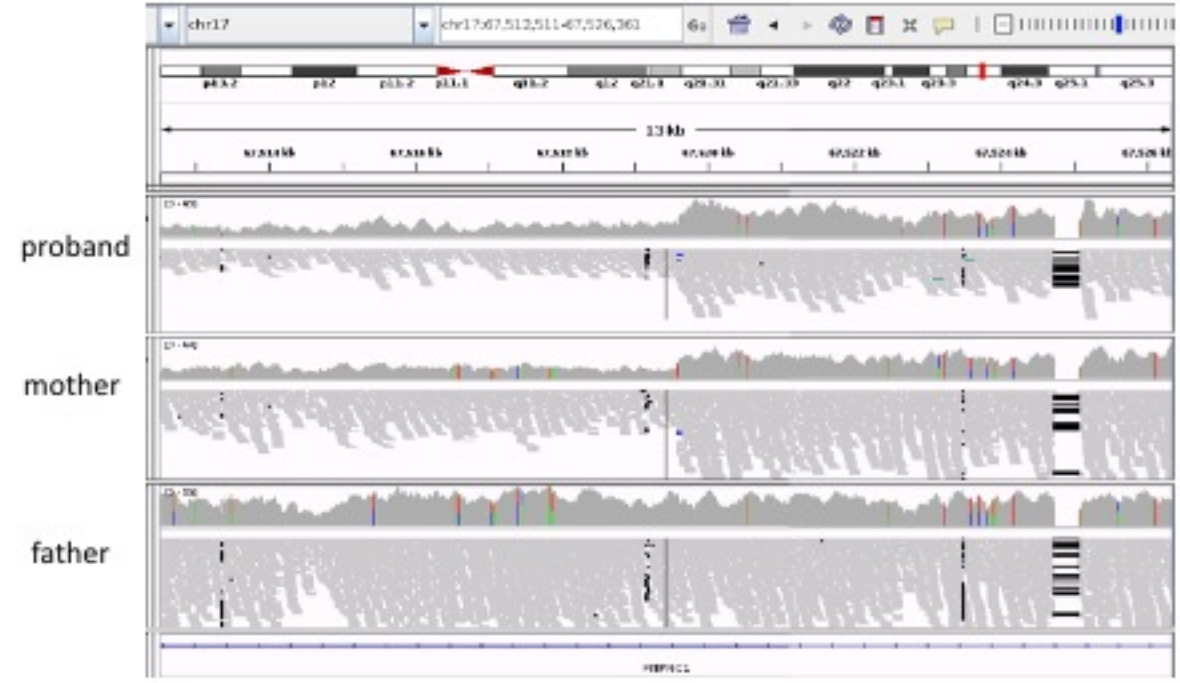
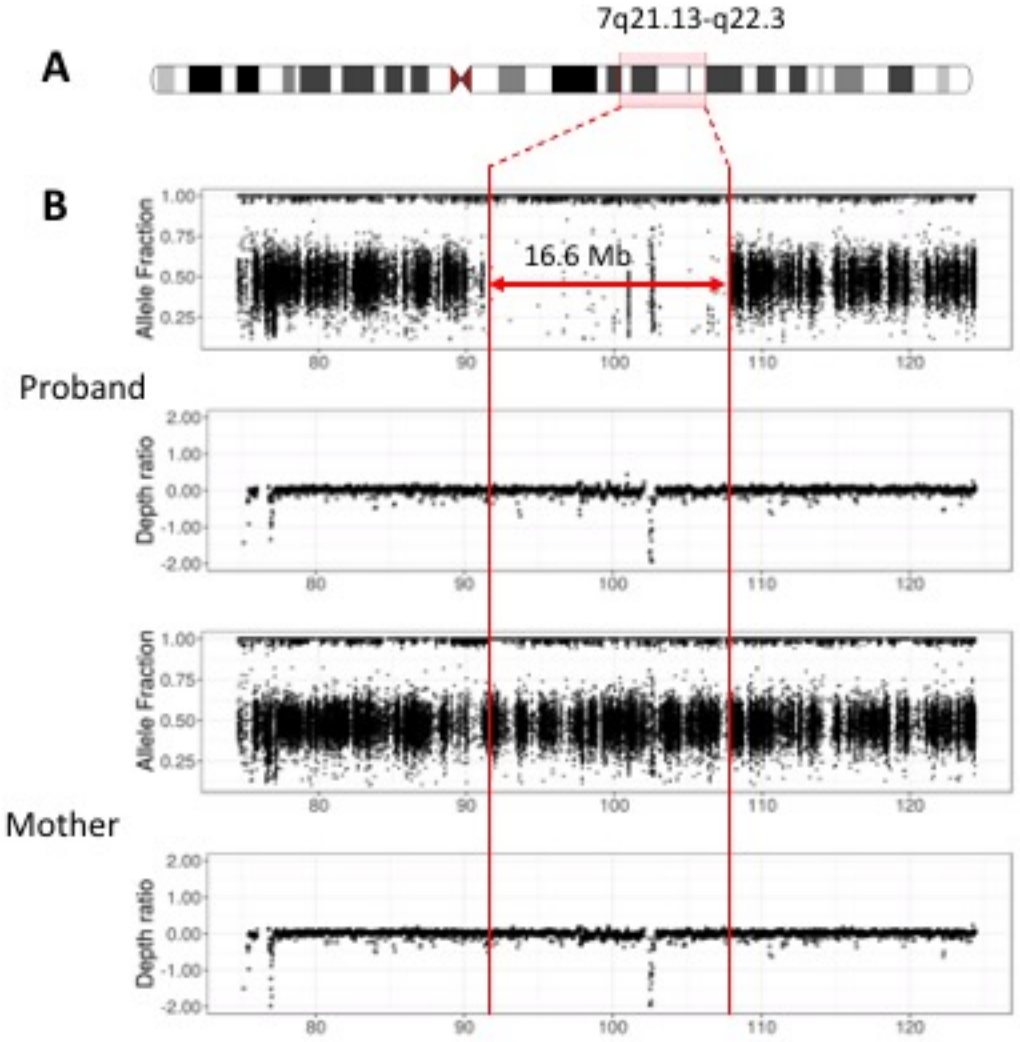
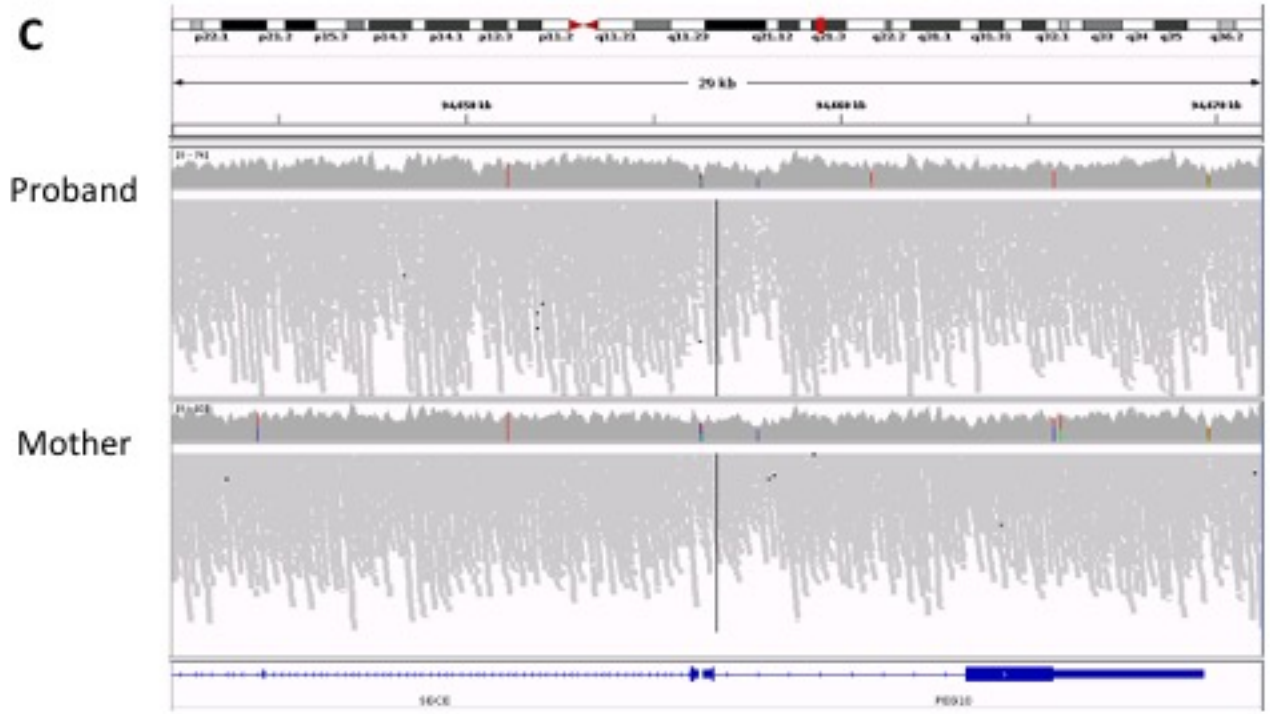


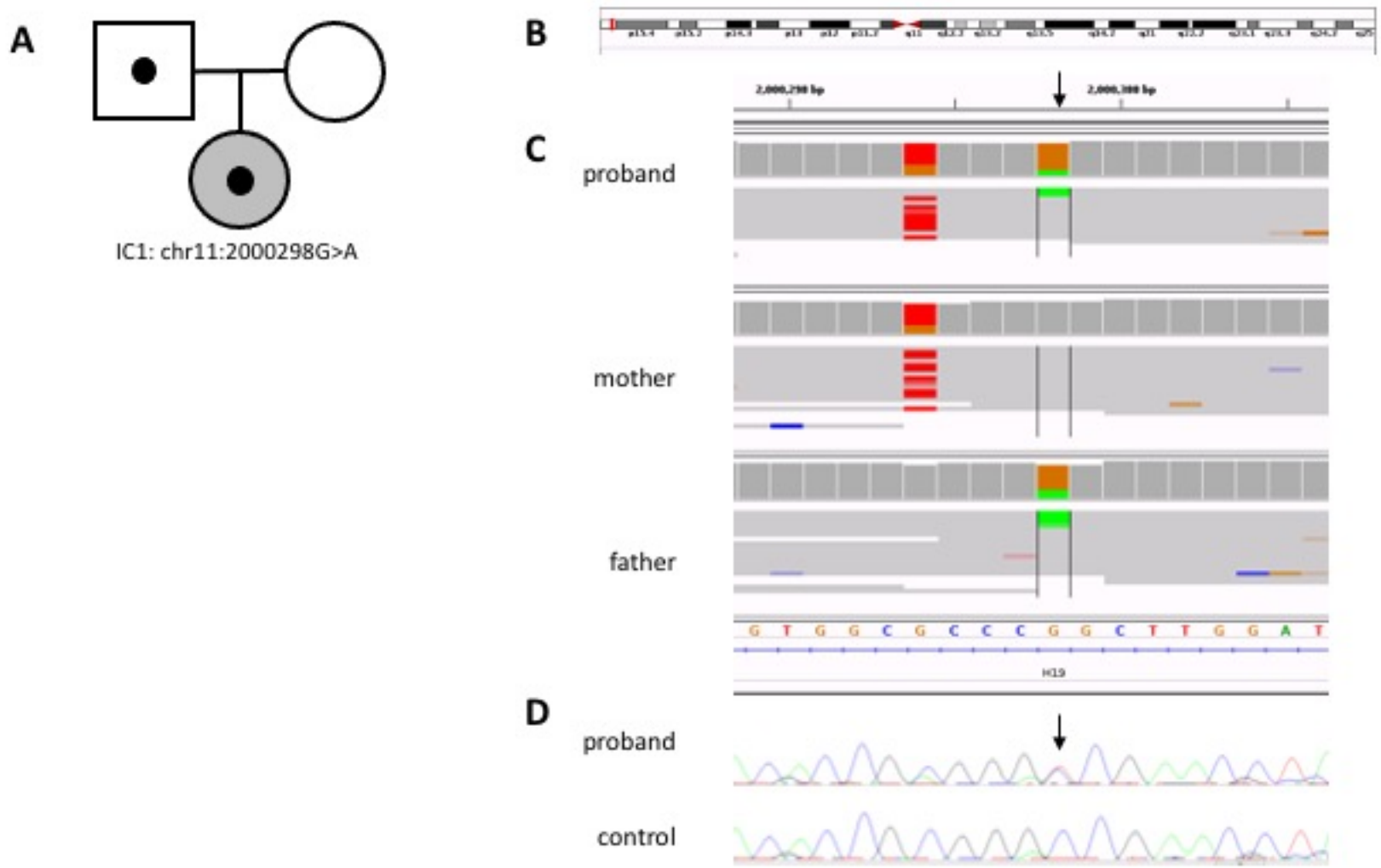


Supplementary Fig 5



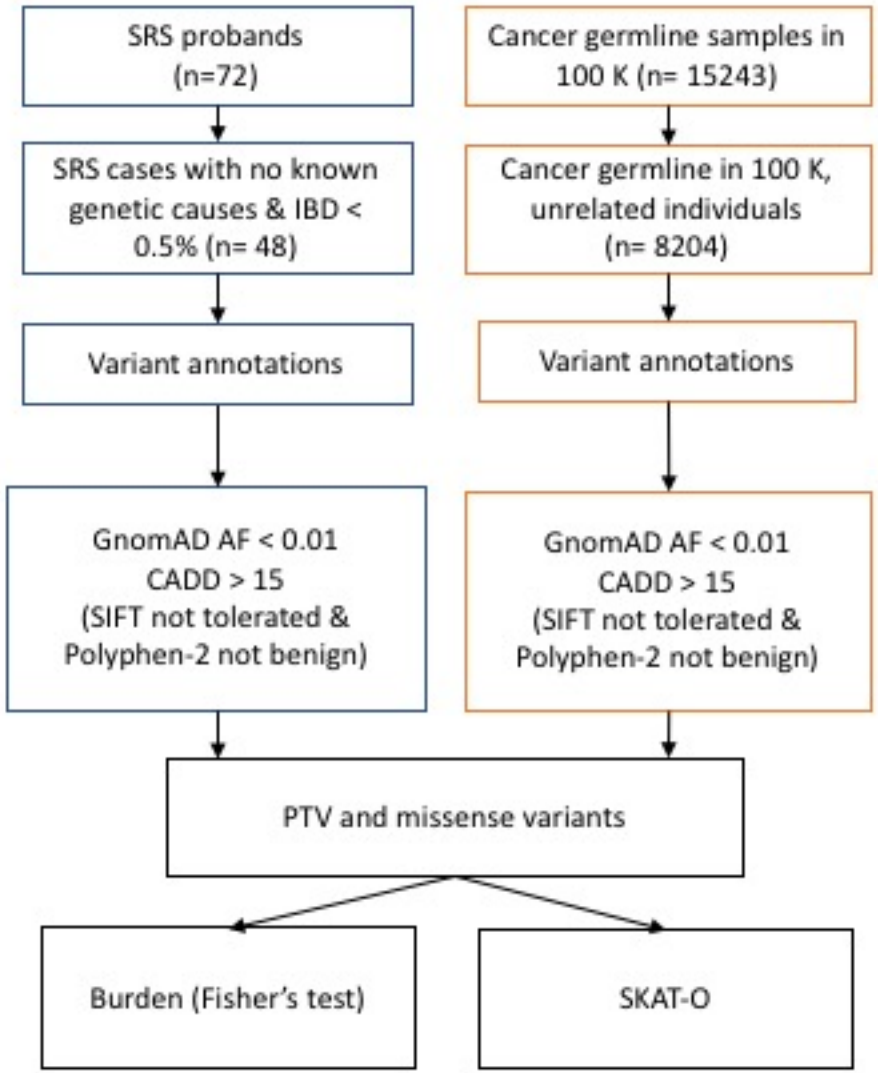


Supplementary Fig 6



Supplementary Figure 7

A



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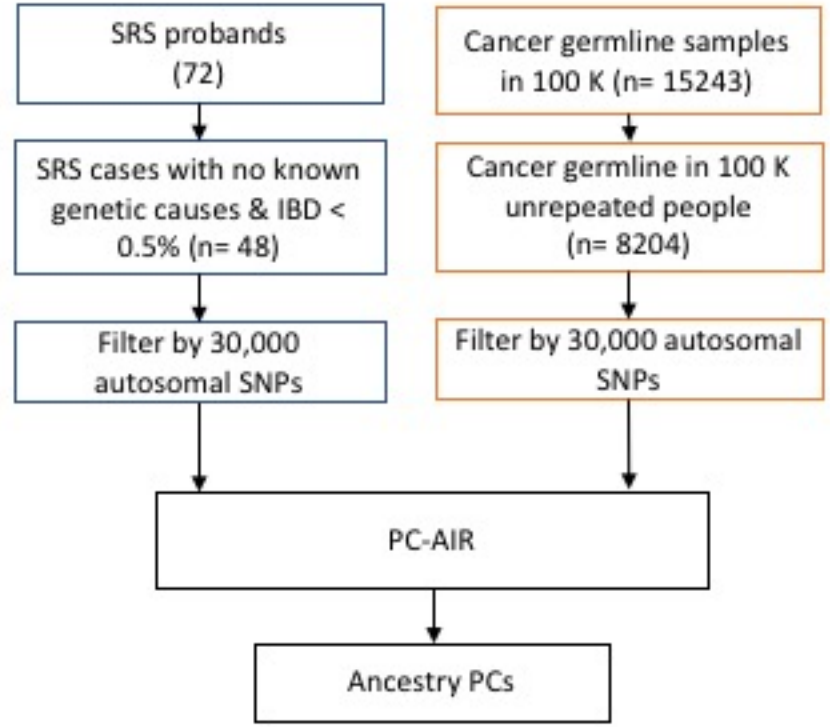

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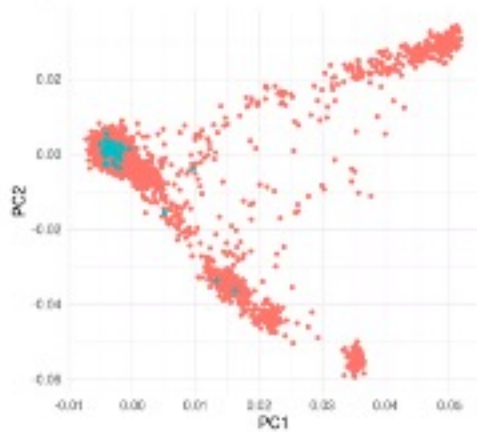

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