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Genetic Testing of Fetal Loss of Heterozygosity Using Single Nucleotide Polymorphism Array and Whole Exome Sequencing

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Article

Keywords: loss of heterozygosity, prenatal diagnosis, single nucleotide polymorphism array, uniparental disomy, whole exome sequencing

Posted Date: August 1st, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3137002/v1

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Abstract

Background

To explore the clinical significance of fetal loss of heterozygosity (LOH), identified by single-nucleotide polymorphism array (SNP array).

Methods

We retrospectively reviewed data form pregnant women who underwent invasivediagnostic procedure at prenatal diagnosis centers in Southeastern China from December 2016 to December 2021. SNP array was performed by the Affymetrix CytoScan 750K array platform. Fetuses with LOH (10 Mb as the threshold or over 5 Mb involving imprinted chromosomes) were further identified by parental verification, MS-MLPA, and/or trio whole exome sequencing (trio-WES), and the genetic results, fetal clinical manifestations, and perinatal outcome were comprehensively analyzed.

Results

Of 11 062 fetuses, 106 (0.96%) with LOH exhibiting a neutral copy number were detected; in 88 (83.0%) of these, LOH occurred in a single chromosome, while 18 (17.0%) fetuses had multiple LOHs on different chromosomes. A total of 66 fetuses had ultrasound anomalies (UAs); the most frequent UA was fetal growth restriction (18/66 (27.3%)). Further genetic analysis was performed in 42 cases (21 cases by parental SNP array verification and 21 cases by trio-WES), in which, we found clinically relevant uniparental disomy in 12 cases, pathogenic variants in five cases, likely pathogenic variants in four cases, variant of unknown significance in six cases, and identity by descent in eight cases. Significantly, the rate of adverse pregnancy outcomes in fetuses with LOH and UAs (24/66 (36.4%)) was higher than in those without UAs (6/40 (15.0%)) ($p< 0.05$).

Conclusions

Fetuses with LOH is not uncommon. Various molecular genetic testing techniques, such as parental SNP array verification, trio-WES, MS-MLPA, regular and systematic ultrasonic monitoring, and the placental study when necessary, should be performed to accurately assess the prognosis of fetal LOH and guide the affected pregnancy management.

Background

In the prenatal diagnostic setting, chromosomal microarray analysis (CMA) improved the diagnostic rate for chromosomal abnormalities by 4.1–10% compared with traditional karyotyping in fetuses with ultrasound anomalies (UA). Single nucleotide polymorphism array technology can not only identify copy number variation (CNV) but also detect chromosome aneuploidy and haploidy, triploidy, loss of heterozygosity (LOH), uniparental disomy (UPD), and low-level mosaicism[1,2].

LOH, referred to as the region of homozygosity in a chromosome, and concerns identity by descent (IBD) and UPD. When LOH is confirmed to have been inherited from only one parent, it is called UPD, which can lead to imprinting disorders involving imprinted chromosomes 6, 7, 11, 14, 15, and 20. Moreover, UPDs were classified as either isodisomy, heterodisomy, or mixed UPD, according to the parental origin. Common mechanisms resulting in UPD include trisomy rescue, monosomy rescue, and somatic mitotic recombination, resulting in mosaic segmental UPD[3,4].

It is challenging to assess the prognosis of fetuses with LOH in prenatal diagnosis. Thus, understanding its clinical significance is necessary due to the phenotypic complexity of LOH and its uncertain pathogenicity. To assess the clinical significance and better understand the correlation between LOH and its phenotype, we investigated the clinical manifestations, performed further molecular genetic analysis using parental SNP array verification, trio whole exome sequencing (WES) and Methylation-specific multiplex ligation dependent probe amplification (MS-MLPA), and tracked the perinatal outcomes of fetuses with LOH.

Patients and methods

Subjects

We retrospectively reviewed pregnant women undergoing invasive diagnostic procedure for a variety of indications at all prenatal diagnosis centers in Fujian Province in southeastern China, Fujian Medical University, China, from December 2016 to December 2021. Most of the cases came from Fujian Maternity and Child Health Hospital. Obtaining informed consent from all the pregnant couples, fetal samples were collected via invasive diagnostic procedure according different weeks of gestation. The study was approved by the Ethics Committee of the Fujian Maternity and Child Health Hospital (No.2016KYLLD01051).

Conventional karyotyping analysis

Karyotyping was performed following the standard cytogenetic protocol, and karyotypes were scanned on Leica GSL120. At least 20 metaphases were counted, and five metaphases were analyzed. Karyotype analysis and description were based on ISCN 2020.

Isolation of genomic DNA

Fetal sample, 15 mg of chorionic villi, 30-40 mL of amniotic fluid, or 2-5 mL umbilical cord blood was obtained, and genomic DNA from the fetus and its parents were extracted using the QIAamp® DNA Blood Mini Kit (Qiagen Inc., Hilden, Germany) following the manufacturer's instructions, and maternal cell contamination was ruled out using microsatellite DNA linkage analysis.

Single nucleotide polymorphism array and data analysis

Chromosomal aberrations, CNVs and LOH were detected using a SNP array on a CytoScan 750K (Affymetrix Inc., Santa Clara, CA) platform containing 200 000 SNPs and 550 000 CNVs probes, all the experimental processes of SNP array were performed as previously described[5]. After obtaining informed consent, parental SNP array verification was performed to confirm the origin of fetal LOH.

The raw data were analyzed using the Affymetrix Chromosome Analysis Suite software (version 3.1.0.15). The coordinate of the chromosome was described based on the genome version hg19. CNVs were classified according to the American College of Medical Genetics (ACMG) guidelines[6]. The reporting threshold was set at CNV ≥ 500 Kb and 10 Mb size as the threshold of LOH or over 5 Mb for a terminal fragment LOH occurred in imprinted chromosomes (chromosome 6, 7, 11, 14, 15 and 20).

The significance of LOH results was interpreted through PubMed (http://www.ncbi.nlm.nih.gov/pubmed), Online Mendelian Inheritance in Man (OMIM; http://www.omim.org/), DECIPHER (https://decipher .sanger.ac.uk/), UCSC (http://genome.ucsc.edu/), ClinGen Dosage Sensitivity Map (https://www.ncbi.nlm.nih.gov/projects/dbvar/clingen/index.shtml), uniparental disomy (http://cs-tl.de/DB/CA/UPD/0-Start.html), Geneimprint (http://www.geneimprint.com/), the Catalogue of Imprinted Genes (www.otago.ac.nz/IGC), the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php), the Locus-Specific Mutation Database (http://www.hgvs.org/dblist/glsdb.htm) and the 1000 Genomes Project Dataset (https://www.ncbi.nlm .nih.gov/variation/tools/1000genomes/).

WES and bioinformatics analysis

To identify homozygous mutations of recessive diseases in addition to UPD, trio-WES (for fetuses and parents) was carried out with the informed consent of pregnant couples. After the sample genomic DNA was extracted, exon capture was conducted using Agilent Sure Select Technology (Agilent, Santa Clara, CA, USA), fragmented randomly, purified, and enriched to construct DNA libraries. Paired-end sequencing was performed on Illumina HiSeq 2500 (Illumina, USA) instruments according to the manufacturer's instructions (Illumina, San Diego, CA, USA).

For sequence alignment, variant calling, and annotation, the sequences were mapped to their location with the human genome reference sequence (hg19 build) using Burrows-Wheeler software (version 0.59)[7]. All SNVs and InDels were annotated with public population frequency databases, including NCBI dbSNP, 1000 Genomes Project, the Exome Aggregation Consortium, as well as OMIM, Swiss-var, Human Gene Mutation Database, ClinVar, and other disease databases, and only variants that were clinically or potentially relevant to the patients' phenotype were reported. Annotation of mutations, prediction of protein function effects and shear harmousness were conducted and the pathogenicity of the variants was assessed according to ACMG[8].

MS-MLPA

Methylation analysis of 7q21.13q36.3 loci was performed by MS-MLPA (SALSA MS-MLPA probe mix ME030-C3 BWS/RSS (MRC Holland, Amsterdam, The Nederland's)), and the relative copy numbers of the three methylation probes in the *MEST* gene (maternal methylation gene region, paternal methylation was preferentially expressed) on chromosome 7q32.2 was determined. All procedures were performed following the manufacturers' protocols performed as previously described[9].

Pregnancy outcome

In fetuses with LOH, we collected data relating to basic information, imaging findings, serological Down's screening results, non-invasive prenatal testing (NIPT) results, results of invasive diagnostic testing, further genetic analysis, perinatal outcomes, and follow-up information. Perinatal outcomes were obtained from delivery records in our hospital. Otherwise, data relating to other cases from other centers were followed up via telephone.

Statistical analysis

SPSS software version 22.0 (SPSS, Inc., Chicago, IL) was used for statistical analysis. Measurement data were expressed as mean ± standard deviation, statistical comparisons were performed using χ^2 test and Fisher's exact test, $p < 0.05$ was considered statistically significant.

Results

Patient characteristics

A total of 11062 fetuses undergoing invasive diagnostic testing over a period of 5 years were analyzed using SNP array, and there were 106 fetuses with LOH. The detection rates of fetal LOH for different invasive diagnostic indications are depicted in Table 1. The mean weeks of gestation and maternal age for pregnancies with fetal LOH was 21±1 (range, 11^{+6} to 31) and 31±3 (range, 19–42) years, respectively. The detailed parental SNP array verification results, trio-WES results, MS-MLPA result, ultrasound findings, and perinatal outcomes of 42 fetuses with LOH are summarized in Table 2, and the data regarding the remaining 64 prenatally diagnosed cases with LOH that declined further genetic testing are listed in Table 3.

Fetal LOH detected by SNP array

The overall flow of fetal LOH analysis is illustrated in Fig. 1. The detection rate of fetuses with LOH ≥10 Mb or ≥5 Mb involving imprinting regions was 0.96% (106/11 062). In 88 (83.0%) fetuses, LOH occurred on a single chromosome, while in 18 (17.0%) fetuses, multiple LOHs were detected on different chromosomes. Of the 18 cases with multiple LOHs, two cases (Cases 1 and 64) were confirmed from consanguineous couples, and the remaining 16 cases denied consanguineous miscarriage.

When LOH occurred on a single chromosome, chromosomes 6 (11.4% (10/88)), 3 (10.2% (9/88)), and 5 (8.0% (7/88)) were the most frequently involved, followed by chromosomes 15 (6.8% (6/88)), 2 (6.8% (6/88)), 8 (6.8% (6/88)), and 13 (6.8% (6/88)) (Tables 2 and 3). LOH identified almost the entire chromosome in seven cases, and the involved chromosomes were chromosomes 2, 6, 13, 14, 18, and 22. In other cases, LOH occurred in a partial fragment of the chromosome, ranging from 5.18 Mb to 96.8 Mb. In total, four cases of mosaic LOH were identified, including in chromosomes 13 and 14, 5q11.1q35.3, and 7q21.13q36.3, respectively. The mosaicism rate ranged from 30% to 80%.

Both cases (Cases 22 and 28) with LOH on the entire chromosome 6 presented with fetal growth restriction (FGR), and were further diagnosed as paternal UPD6 and maternal UPD6, respectively. Finally, both patients elected termination of pregnancy (TOP) (Table 2). Isolated segmental LOH on chromosome 6 was identified in 10 cases, of which nine presented with UAs, including FGR, thickened nuchal translucency (NT), enhanced bowel echo, intracardiac echogenic focus, increased umbilical artery resistance index, oligohydramnios, cervical lymphatic hygroma, mild regurgitation of tricuspid valve, fetal bilateral renal enlargement, increased renal echogenicity, reverse a-wave of ductus venosus, and enhanced intestinal echo, resulting in TOP (*n*=6) and preterm birth (*n*=1). The other three cases had a favorable outcome.

Isolated LOH on chromosome 3 was identified in nine cases, of which only two presented with UAs, including thickened NT, resulting in abortion (*n*=1). The other seven cases showed no anomalies on prenatal ultrasound and had no obvious abnormal phenotypes after birth.

Isolated fetal LOH on chromosome 5 was identified in seven cases, of which three all had abnormal ultrasound findings, including FGR, small fetal head circumference (HC) for gestation age, lethal bone dysplasia (osteogenesis imperfecta type II), micrognathia, small biparietal diameter (BPD), HC, and femur length (FL) for gestation age, bilateral femoral curvature, less than the normal predictive value -2SD, hydrops fetalis, fetal giant bladder, fetal lung cystic adenoma, resulting in TOP (n=3) or preterm birth (n=1), and missed abortion (n=2); the other case (Case 34) showed minor abnormal phenotypes on prenatal ultrasound, and presented micrognathia after birth.

UAs in fetal LOH detected by SNP array

Totally, 66 fetuses presented UAs, including 22 (33.3%) with structural abnormalities, 24 (36.4%) with ultrasonic soft marker, 18 (27.3%) with FGR and 9 (13.6%) with other presentations. The most common soft marker anomaly was thickened NT. The most frequent ultrasonic structural anomalies were cardiovascular (9.1%), skeletal (6.1%), and genitourinary malformations (6.1%). In the group with UAs, fetal LOH mostly involved chromosomes 5, 6, and 1, whereas LOH mostly occurred on chromosomes 3, 10, and 18 in the group without UAs (Tables 2 and 3).

FGR was detected prenatally in 18 fetuses, of which 11 had FGR as an isolated ultrasound finding. The most frequently involved chromosome was chromosome 6 (n=6) in cases with FGR, followed by chromosomes 7 (n=3) and 17 (n=3). The outcomes of these 18 fetuses included TOP (n=11), preterm birth (n=3), and term birth (n=4). The incidence of fetal TOP was significantly higher in fetuses with FGR than in those without FGR (61.1% (11/18) *vs* 21.6% (19/88), $p < 0.01$) (Tables 2 and 3).

UPD results

To verify the parental source of fetal LOH, 21 cases of fetal LOH were confirmed by parental SNP array analysis, of which two (9.5%) had paternal UPD (Cases 4 and 22) and 12 (57.1%) had maternal UPD, namely, case 4 (UPD 14 pat), case 6 (UPD7 mat), case 14 (mosaic UPD7 mat), case 19 (UPD15 mat), case 21 (UPD 16 mat), case 22 (UPD6 pat), case 24 (UPD15 mat), case 25 (UPD11 mat), case 26 (UPD7p22.3p12.2 mat), and case 30 (UPD11 mat); four cases were confirmed to be IBDs (Cases36-42). Among the 14 cases with UPD, cases 19 and 24 were diagnosed with Prader–Willi syndrome (PWS), cases 6, 14, 25, 26, and 30 were determined Silver-Russell syndrome (SRS), cases 4 and 22 were diagnosed with Kagami-Ogata syndrome (KOS) and transient neonatal diabetes mellitus, respectively. None of confirmed imprinted genes were detected in the cases 20, 23, 27, and 28 except case 21 (UPD16 mat), thus classifying them variant of uncertain significance (VOUS). Among the 14 UPDs, notably, in case 14, amniocentesis was performed due to fetal right aortic arch with aberrant left subclavian artery as ultrasound indicated, LOH with a size of 70.4 Mb was observed in 7q21.13q36.3 by SNP array (Fig. 2A). First, parental SNP array verification indicated that it is not possible to determine whether source of the LOH on chromosome 7 is paternal or maternal (Fig. 2B); Then, MS-MLPA for the methylation analysis of 7q21.13q3615 loci revealed that the relative copy numbers of the three methylation probes (184 bp, 190 bp and 256 bp) in the *MEST* gene (maternal methylation gene region, paternal methylation was preferentially expressed) were 0.66, 0.66, and 0.64, respectively, suggesting a possible low proportion mosaic maternal UPD7 associated with Silver-Russell syndrome (Fig. 2C). However, the experimental result was close to the threshold range; thereby, it could not be determined and interpreted accurately. The fetus was term delivered, feeding difficulties existed after birth, 3 months after delivery at present, the child's height and development were normal, only light weight (Table 2).

Twenty-one cases of fetal LOH were further verified by trio-WES, of which one (4.8%) had paternal UPD and four (19.0%) had maternal UPD. Notably, trio-WES was performed in case 12, and maternal UPD15 associated with PWS was confirmed, and the pregnancy was terminated at 28 weeks (Table 2). None of the definite imprinted genes observed in the other four UPDs, thus classifying a VOUS.

Karyotyping results

Karyotyping was performed successfully in all 106 fetuses with LOH. In total, 97 cases yielded normal results, and nine cases had abnormal karyotyping results, including 47,XX,+mar dn, 46,X,i(Xq)[40]/45,X[14], 46,XX,t(4;5)(q31;q23)pat, 46,XY,t(11;22)(q24.1;q12.3) dn, 47,XX,+21, mos 47,XY,+22[3]/46,XY[58], 46,XX,t(12;21)(q12;q22.2) mat, 47,XN,+mar[57]/46,XN[41] and 46,X,inv(Y)(p11.2q11.2) mos 45%.

Gene mutation results

Trio-WES was performed in 21 cases to detect gene mutation of autosomal recessive diseases in addition to UPD, and 11 results were clinically significant, including 5 pathogenic variants, 4 likely pathogenic variants and 2 pathogenic UPDs. Among these clinically significant results, three cases (Cases 1, 9, and 15) were identified homozygous mutations in autosomal recessive diseases attributed to LOHs by trio-WES.

For case 11, fetal BPD was small for gestation age by ultrasound, SNP array showed a 38 Mb LOH in 14q13.1q24.2, prenatal trio-WES declined, the fetus was term birthed, the infant was 5 months old now, 60 cm tall, and often arches her back; brain MRI at three months of age revealed that bilateral ventricles were asymmetrical, the left lateral ventricle was larger than the right, some of the extracerebral spaces are slightly widened. Low T1W1 and high T2W1 signals were observed in the bilateral maxillary, ethmoid, and sphenoid sinus. Postnatal trio-WES indicated UPD14 was ruled out, and showed a de novo heterozygous mutation, NM_000095: c.1417_1419dup (p. D473dup), in *COMP* in the female infant (Fig. 3), which is an incidental finding, associated with autosomal dominant pesudoachondroplasia (PSACH, OMIM:177170), epiphyseal dysplasia, multiple, 1(EDM1, OMIM:132400), and carpal tunnel syndrome 2 (CTS2, OMIM:619161). For case 18, amniocentesis was performed, as the fetal left femur was slightly curved, and the SNP array revealed a 20 Mb LOH located in 5q23.2q32. Prenatal trio-WES indicated a de novo missense variant, NM_000088: c.1436G>C p.G479A , in *COL1A1* (120150) on chromosome 17 in the fetus (Fig. 4), associated with osteogenesis imperfecta, type I (OMIM:166200), type II (OMIM:166210), type III(OMIM:259420), type IV (OMIM:166220), Ehlers-Danlos syndrome, arthrochalasia type 1 (OMIM:130060), Caffey disease (OMIM:114000), and bone mineral density variation QTL, steoporosis (OMIM:166710), and the couple elected TOP at 26 weeks. In case 29, amniocentesis was performed, as the fetal bilateral femoral curvature was less than the normal predictive value -2SD was detected on ultrasound, and the SNP array revealed a 46.1 Mb LOH located in 5p15.33p11. Prenatal trio-WES indicated an inherited paternally splicing variant, NM_000088.4: c.1615-1G>T (p. G802V), in *COL1A1* (120150) on chromosome 17 in the fetus (Fig. 5), associated with autosomal dominant osteogenesis imperfecta, and the pregnancy was terminated at 23 weeks (Table 2).

Perinatal outcome and follow-up

The pregnancy outcomes of the 106 fetuses with LOH were as follows: 67 term births (of which two resulted in neonatal death, and one had an abnormal phenotype after birth), 29 TOPs, three preterm births, two fetal deaths, and five miscarriages. A full-term infant (Case 14) was diagnosed with feeding difficulties that existed after birth, and 3 months after delivery, the child's height and development were normal, with only low weight. A preterm infant (Case 2) was diagnosed with autism postnatally. One fetus (Case 11) showed fetal BPD that was small for gestation age on prenatal ultrasound screening and was delivered vaginally at term. The infant showed growth delay (60 cm tall at 5 months old), hypotonia, and often arched her back. The rate of adverse pregnancy outcomes in fetuses with LOH with UAs was 52.4% (22/42), while it was 32.8% (21/64) in those that did not show positive ultrasound findings ($p < 0.05$).

Discussion

In our cohort, we investigate the clinical significance of fetal LOH as well as the correlation between fetal LOH and its clinical features. The rate of LOH meeting the report threshold in our study was 0.96%, slightly lower than the 0.97% reported by Liu et al.[10], which might be attributed to different sample sizes of the study population and the threshold of LOH reported. The threshold in our cohort was set according to the reported study by Hoppman, et al.[11]. In addition, none of clinically significant LOHs occurring in chromosome X were reported due to lack of the adverse family history associated with X-linked disorders. Thereby fetal LOH occurred on a single chromosome, chromosomes 6 and 5 were the most common involved; whereas, Liu et al.[10] showed that LOH was more likely to occur in chromosomes X, 2 and 16. The discordance may be due to the reporting threshold of the fetal LOH studied.

Clinically significant imprinting disorders should be valued, especially for UPD involving imprinted chromosomes 6, 7, 11, 14, 15 and 20[12,13]. The clinical significance of UPD is closely associated with the affected imprinted region and genes in addition to parental origin [14,15]. It is unclear whether there is an imprinting effect in UPD 16[16–18], the outcomes of the carriers were variable, from normal growth to delayed growth[18]. Notably, in cases 21 and 27, pregnancies confirmed with maternal UPD16 were terminated owing to UAs and abnormal genetic results.

In our cohort, 62.3% (66/106) of fetuses with LOH presented UAs, and the most common UA was FGR or FGR combined with other indications (18/66 (27.3%)), and the most frequently ultrasound structure anomalies being cardiovascular system malformations (6/66 (9.1%)), skeletal malformations (4/66 (6.1%)), and genitourinary malformations (4/66 (6.1%)). Seventeen percent (18/106) of fetuses with LOH were FGR, UPDs 2, 7, 14, 15, and 16 were the underlying genetic causes of FGR [12,19,20]. The possible pathogenesis encompasses homozygous pathogenic variants in single gene diseases, imprinting effect, or confined placental mosaicism (CPM) [21]. Thus, UPD is one of the possible genetic factors resulting in FGR. Monitoring fetal growth via ultrasound is essential for the management of fetal LOH. Indicative prenatal ultrasound findings can be observed in patients with Beckwith-Wiedemann syndrome and SRS[22,23]. In our cohort, four cases (Cases 6, 25, 26 and 30) with LOH showed FGR or FGR combined with other indications, of which four UPDs were confirmed. The genetic causes underlying FGR were maternal UPD7 and maternal UPD11 associated with SRS[12], and TOP was elected owing to unfavorable outcomes. Patients with UPD14 showed multiple UAs, resulting in unfavorable outcomes[24,25]. Paternal UPD14 associated with KOS was confirmed in case 4 with polyhydramnios (amniotic fluid index: 38.7 cm), the pregnancy was terminated finally. Notably, the rate of adverse pregnancy outcomes in fetuses with LOH and UAs (36.4%) was higher than in those without UAs (15.0%) ($p < 0.05$), our data demonstrate that it is essential that regular ultrasound screening should be examined to closely monitor the development for fetuses with LOH.

LOH also provides certain signs for investigating homozygous variants in autosomal recessive single gene diseases besides UPD and imprinting effects. In three cases in our cohort, pathogenic homozygous variants in single gene diseases were further identified via trio-WES, resulting in UAs. Thereby, trio-WES should be first performed for its ability of UPD verification as well as identifying homozygous mutations simultaneously (Cases 11, 16, and 17). Furthermore, six cases (Cases 2, 11, 16, 17, 18, and 29) with clinically significant variant were detected, which were incidental findings. Our study also shows that trio-WES

could identify incidental pathogenic mutations in addition to homozygous variants attributed to LOH. Thereby, Trio-WES should be recommended first for fetal LOH, especially in fetuses with structural anomalies and/or consanguineous parents.

Our cohort had some limitations. First, although it was a retrospective multicenter study, the sample size was not large enough, studies with larger population will be needed, and the follow-up period was not long enough, which might miss some clinical features. Second, the parental origin of LOH was further identified in only 39.6% of cases and none of the cases were further investigated in placental tissues to confirm CPM.

Conclusion

We explore the clinical significance and features of fetal LOH. Various molecular genetic testing techniques, such as parental SNP array verification, trio-WES, MS-MLPA, regular and systematic ultrasonic examination, and placental study when necessary, should be comprehensively performed to precisely assess the prognosis of fetal LOH and guide the affected pregnancy management.

Declarations

Acknowledgments

We thank the family members who participated in our study. We also appreciate the obstetricians, radiographers, sonographer and pediatricians who offered assistance to our study.

Author contributions

HX, LZ and AY prepared the main manuscript; XC, LX, ML, NL, QG, LC, and HH prepared the experiment. All authors have read and approved the final article.

Funding

This study was sponsored by the Joint Funds for the Innovation of Science and Technology, Fujian Province (no.2020Y9149), 2021 Fujian provincial health technology project (no.2021GGA051), and Natural Science Foundation of Fujian Province (no.2022J01421).

Availability of data statement

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of interest

The authors confirm that there are no conflicts of interest.

Ethics approval and consent to participate

The study complied with the principles set forth in the Declaration of Helsinki. It was approved by the Institutional Review Board of Fujian Maternal and Child Health Hospital. Written informed consent was obtained from each patient.

Consent for publication

Acknowledgements Informed consent was obtained from all patients for being included in the study.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1 The detection rate of fatal LOH by SNP array for different invasive testing indications

Indication for invasive testing	Case number	Fetal LOH detected
	N	n (%)
Ultrasonic structural anomalies	2749	23 (0.84）
Other ultrasound anomalies	4830	47 (0.97）
Positive NIPT result	1462	10 (0.68）
High risk for Down's screening	376	6 (1.86)
Parental abnormal karyotype	385	1 (0.26)
Consanguinity	15	6 (6/15*)
Previous adverse pregnancy	705	3 (0.43)
Advanced maternal age	540	14 (2.59)
Total	11062	106 (0.96)

Data are given as *n* (%).

Each case was classified based on most important indication. Classification of indications arranged from most to least important was as follows: ultrasound structural anomalies, fetal karyotype abnormality, soft marker, positive NIPT results, high risk for Down's screening, parental genetic factors, consanguinity, previous adverse pregnancy, AMA and others.

* If cases number is less than 20, the percentage is not calculated.

#Other ultrasound anomalies included soft-marker anomalies, FGR or fetal overgrowth, hydramnios, hydramnios and abnormal blood flow on doppler ultrasound.

LOH, loss of heterozygosity; NIPT, non-invasive prenatal testing; SNP, single nucleotide polymorphism

Table 2 Forty-two fetuses with LOH accepted further genetic testing

Case	GW	Prenatal ultrasound findings/Invasive testing indication§	Fetal karyotype	Fetal LOH region (hg 19) detected by SNP-array	Size (Mb) /Percentage (%)*	Results of further genetic testing (classification of pathogenicity)
1[a]	22	Oligohydramnios, strephenopodia, consanguineous marriage	46, XX	1p36.33p36.21(888,658-14,505,595) x2 hmz 2q11.1q14.3(95,550,957-127,459,321) x2 hmz	13.6 Mb 31.9 Mb (3.69%)	Prenatal trio-WES indicated a homozygous mutati NM_000302: c.2071_2072 ins CC (p.R693Qfs*122), in *PLOD1* (153454) in t fetus, associated wit Ehlers-Danlos syndrome type 1 (OMIM 225400) whi was located in the L of 1p36.33p36.21, a both parents were heterozygous for thi variation (LP)
2	21	Decreased fetal FL/BPD and FL/HC ratio	46, XY	12q23.3q24.32(106,008,417-126,307,790) x2 hmz	20.3 Mb	Postnatal trio-WES indicated a heterozygous mutation, NM_0013 c.45+1G>C, in *DDX3* which is associated with intellectual developmental disorder, X-linked, syndrome, Snijders Blok type (MRXSSB, OMIM:300958) (P)
3	19+	FGR intracardiac echogenic focus, increased umbilical artery resistance index, oligohydramnios	46, XX	6p25.3p23(203,878-13,411,320) x2 hmz 6p21.1p11.1(41,305,454-58,726,706) x2 hmz 6q11.1q14.1(61,972,918-75,972,465) x2 hmz 6q22.31q25.1(123,041,062-149,830,858) x2 hmz (containing imprinted genes *PLAGL1* (603044) and *HYMAI* (606546))	13.2 Mb 17.4 Mb 13.9 Mb 26.7 Mb	Prenatal trio-WES indicated mix UPD (mat (P)
4	26+3	Polyhydramnios (AFI 38.7 cm)	46, XY	arr (14)x2 mos hmz (80%)	Mosaicism 80%	Parental SNP array verification indicate UPD (14) pat, which associated with Kagami-Ogata syndrome (P)
5	24	Multiple malformations, thickened prenasal skin, "fish mouth" shape mouth	46, XX	1p36.11p21.2(26,044,678-99,969,487) x2 hmz 3p25.3p14.2(8,746,546-60,897,120) x2 hmz 6p25.1p22.1(5,596,983-28,449,315) x2 hmz 6q24.2q25.2(143,241,806-155,371,652) x2 hmz 7p14.3p11.1(33,991,108-58,019,983) x2 hmz 7q11.21q22.1(62,569,501-98,898,149) x2 hmz 11p15.1p11.12(17,372,347-51,550,787) x2 hmz 11q11q14.1(54,827,207-84,312,637) x2 hmz	73.9 Mb 60 Mb 22.9 Mb 12.1 Mb 22.8 Mb 24 Mb 34.2 Mb 29.5 Mb (10%)	Postnatal trio-WES indicated a homozygous mutati NM_173076.3: c.6577_657 del, in *ABCA12* in the fetus, associated with autosomal recessive congenital ichthyosi type 4B type (OMIM 242500) and 4A typ (OMIM 601277) (P)
6	13^{+6}	FGR	46, XY	7q31.31q36.3(120138084_159118443) x2 hmz	39 Mb	Parental SNP array verification indicate UPD (7) mat, which i associated with Silv Russell syndrome (P
7	18^{+}	AMA	46, XX	2q22.3q31.1(144,624,648-173,171,481) x2 hmz	28.5 Mb 23.5 Mb	Prenatal trio-WES indicated no clinical relevant mutations.

				4q13.1q21.3(63,730,113-87,252,596) x2 hmz		
8	23^{+}	Small fetal BPD and HC for gestation age	46, XX	14q32.11q32.31(91556694_101593701) x2 hmz	10 Mb	Prenatal trio-WES indicated no clinical relevant mutations.
9	24	Fetal ARSA	46, XY	1p36.21p35.2(15,728,288-31,781,279) x2 hmz 4p15.2p11(25,981,952-49,063,479) x2 hmz	16 Mb 23 Mb	Prenatal trio-WES indicated no clinical relevant mutations., and identified a homozygous mutati NM_015378: c.427A (p.R143G), in *VPS13* in the fetus, associa with autosomal recessive spinocerebellar, atax autosomal recessive (SCAR4, OMIM 607317) (VOUS)
10	25	Fetal bilateral renal enlargement, increased renal echogenicity	46, XY	8q11.23q24.3(55365228_146292734) x2 hmz	90.9 Mb	Prenatal trio-WES indicated UPD (8q11.23-q24.3) mat (VOUS)
11	26	Small fetal BPD was small for gestation age, consanguineous marriage	46, XX	14q13.1q24.2(34435418_72618432) x2 hmz	38 Mb	Postnatal trio-WES confirmed no indication of UPD (1 and showed a heterozygous mutation, NM _0000 c.1417_1419 dup (p.D473dup), in *COM* in the female infant, associated with autosomal dominan pesudoachondropla (PSACH, OMIM177170), epiphyseal dysplasia multiple, 1 (EDM1,OMIM 13240 and carpal tunnel syndrome 2 (CTS2, OMIM 619161) (LP)
12	22	FGR; high risk of trisomy 15 detected by NIPT; AMA	46, XY	15q21.3q26.1(53188649_90583138) x2 hmz	37.4 Mb	Prenatal trio-WES indicated mixed UPD (15q21.3q26.1) mat associated with PWS (P)
13	18	Thickened nuchal translucency (2.8 mm), cervical lymphatic hygroma	46, XX	6p12.2p11.1(52607147_58726706) x2 hmz, 6q11.1q12(60972918_66615551) x2 hmz	6.1 Mb 5.6 Mb	Prenatal trio-WES indicated no clinical relevant mutations.
14	25	Fetal RAA-ARSA	46, XY	7q21.13q36.3(88,712,610-159,118,443) x2 mos hmz (45%)	70.4 Mb	First, parental SNP array verification indicated it is not possible to determin whether the LOH of chromosome 7 is paternal or maternal

						Then, MS-MLPA for methylation analysis of 7q21.13q36.1 loc MS-MLPA revealed t the relative copy numbers of the three methylation probes (184 bp, 190 bp, and 256 bp) in *MEST* ge (maternal methylatio gene region, paterna methylation was preferentially expressed) were 0.6 0.66 and 0.64, respectively, suggesting a possib low proportion mosa UPD (7) mat. Howev the experimental results are near the threshold range, so i cannot be interprete accurately (P)
15	23+	Fetal bilateral renal enlargement, increased renal echogenicity	46, XX	6p12.3q14.1(46587519_77260358) x2 hmz	27.45 Mb (0.95%)	Prenatal trio-WES revealed a homozygous mutati NM_138694.4: c.1233G>A p.K411K, *PKHD1* in the fetus, associated with autosomal recessive polysystic kidney disease 4, with or without hepatic Disease (OMIM 263200) (P)
16	21+	Reproductive history of children with chromosomal abnormalities (arr[hg19]9p24.3p22.3(203861_16540793) x1, 9p22.3p13.3(16540940_34942483) x3, which is associated with 9p partial monosomy syndrome and 9p trisomy, respectively; AMA	46, XY	18p11.32p11.21(136305_15079294) x2 hmz 18q11.1q23(18552517_77997606) x2 hmz	15 Mb 59 Mb	Prenatal trio-WES indicated UPD(18) p and also revealed a heterozygous splicin mutation, NM_006767.4: c.2069+1G>A, in *LZTR1* in the fetus, associated with noonan syndrome 2 (NS10, OMIM 61656 and schwannomato 1, susceptibility, (OM 615670), inherited paternally (LP)
17	18+	NIPT indicated a 8.2 Mb deletion at 4q31-qter (FF: 6.4 %)	46, XY	4q32.3q35.2(167230247_190921709) x2 hmz	23.7 Mb	Trio-WES indicated i UPD (4q32.3q35.2) mat [(UPD (4)], and also revealed a heterozygous splicin mutation, NM_001807.6, in *CE* in the fetus, associa with maturity-onset diabetes of the youn type VIII (OMIM 609812), inherited paternally (LP)
18	20	Lethal bone dysplasia (osteogenesis imperfecta type II)	46, XX	5q23.2q32(125,771,613-145,714,232) x2 hmz	20 Mb	Prenatal trio-WES indicated a de novo missense variant, NM_000088: c.1436G>C p.G479A *COL1A1* (120150) o chromosome 17, associated with osteogenesis imperfecta, type I (OMIM166200), type (OMIM 166210), typ (OMIM 259420), typ IV (OMIM 166220), Ehlers-Danlos syndrome, arthrochalasia type (OMIM 130060), Caffey disease (OMI

						114000), and bone mineral density variation QTL, steoporosis (OMIM 166710) (P)
19	28+	FGR	46, XX	15q14q21.3(35,077,111-54,347,324) x2 hmz	19.2 Mb	Parental SNP array verification indicate UPD (15) mat, associated with PW (P)
20	27+	Narrow inner diameter of aortic arch, increased renal echogenicity, FGR, persistent left superior vena cava, thickening of the placenta	46, XY	2p25.3p11.2(50,813-87,053,152) x2 hmz 2q11.1q37.3(95,550,957-242,773,583) x2 hmz	82.0 Mb 147.2 Mb	Parental SNP array verification indicate UPD (2) mat (VOUS)
21	23+	VSD, FGR, aortic stenosis, left kidney dysplasia or absence, enhanced intestinal echo	46, XX	16q23.2q24.3(79,800,878-90,146,366) x2 hmz 16p13.3p12.3(94,807-19,302,326) x2 hmz	10.3Mb 19.2Mb	Parental SNP array verification indicate UPD (16) mat (P)
22	22+	FGR, mild tricuspid regurgitation, reverse a-wave of ductus venosus, enhanced intestinal echo	46, XY	6p25.3q27(203,877-170,896,644) x2 hmz	170.7 Mb	Parental SNP array verification indicate UPD (6) pat, associated with transient neonatal diabetes (P)
23	19	Amniocentesis: 47, XX, +mar dn; high risk of trisomy18 screening	47, XX, +mar dn	3p13p12.1(72,069,483-86,073,653) x2 hmz 3q11.2q22.3(95,320,713-137,634,506) x2 hmz	14.0 Mb 42.3 Mb	Parental SNP array verification indicate UPD (3) mat (VOUS)
24	29	Sever FGR	46, XX	15q11.2q22.2(22,817,870-62,568,746) x2 hmz 15q26.2q26.3(96,143,558-102,397,317) x2 hmz	39.7 Mb 6.25 Mb	Parental SNP array verification indicate UPD (15) mat, associated with PW (P)
25		FGR, high risk of trisomy 21 screening	46, XY	11p15.5p15.4(205,827-8,150,933) x2 hmz	8 Mb	Parental SNP array verification indicate UPD (11) mat, associated with Silv Russell syndrome (P
26	30	Severe FGR	46, XX	7p22.3p12.2(50944_157155880) x2 hmz	50.4 Mb	Parental SNP array verification indicate UPD (7p22.3p12.2) mat, associated with Silver-Russell syndrome (P)
27	23	Fetal HC was less than the mean 3SD; high risk of trisomy 16 detected by NIPT (Z=18.705)	46, XX	16q22.3q24.3(73705779_90146366) x2 hmz	16 Mb	Parental SNP array verification indicate mixed UPD (16) mat (VOUS)
28	25	FGR; high risk of trisomy 16 detected by NIPT (Z=25.2); AMA,	46, XY	6p25.3q27(203,877-170,896,644) x2 hmz	170.7 Mb	Parental SNP array verification indicate mixed UPD (6) mat (VOUS)
29	23	Fetal bilateral femoral curvature, less than the normal predictive value -2SD	46, XY	5p15.33p11(113,576-46,242,541) x2 hmz	46.1 Mb	Prenatal trio-WES indicated an inherite paternally splicing variant, NM_000088 c.1615-1G>T (p. G802V), in *COL1A1* (120150) o chromosome 17, associated with autosomal dominan osteogenesis imperfecta (P)
30	29	Hydrops fetalis, FGR	46, X, i(Xq) [40]/45, X[14]	11p15.5p15.4(230,750-8,050,928) x2 hmz	7 Mb	Parental SNP array verification indicate UPD (11) mat, associated with Silv Russell syndrome (P

31	26	FGR, high risk of trisomy 21 screening	46, XX	7q11.21q11.23(62569502_75233244) x2 hmz 7p14.1p11.1(42421781_58019983) x2 hmz 9q21.13q22.2(78780578_92509057) x2 hmz	12.7 Mb 15.6 Mb 13,7 Mb	Prenatal trio-WES indicated no clinical relevant mutations.
32	18	High risk of trisomy 21 screening	46, XY	7q22.3q31.32(107306055_122301501) x2 hmz	15 Mb	Prenatal trio-WES indicated no clinical relevant mutations.
33	19	Deep notch a-wave of ductus venosus	46, XX	13q14.2q21.2(47358540_61986828) x2 hmz	14.6 Mb	Prenatal trio-WES indicated no clinical relevant mutations.
34	25	Fetal micrognathia, small BPD, HC and FL for gestation age	46, XY	5q15q22.1(94849287_110237725) x2 hmz	15.39 Mb	Prenatal trio-WES indicated no clinical relevant mutations.
35	23	Fetal suprachivalal stenosis	46, XX	1p31.3p31.1(65532190_78558941) x2 hmz	13.03 Mb	Prenatal trio-WES indicated no clinical relevant mutations.
36	22	Fetal pulmonary sequestration	46, XX	2p24.3p21(16,435,384-42,306,722) x2 hmz	25.8 Mb	Parental SNP array verification indicated IBD
37	18	Thickened nuchal translucency (3.5 mm); AMA	46, XY	14q11.2q12(22,094,953-32,626,332) x2 hmz	10.5 Mb	Parental SNP array verification indicated IBD
38	19	Pregnant women had a child with mental retardation; AMA	46, XX	3p22.1p14.2(42390369_60178833) x2 hmz	17.7 Mb	Parental SNP array verification indicated IBD
39	18	Previous adverse pregnancy	46, XY	15q21.2q22.2(49971494_63093257) x2 hmz	13.1 Mb	Parental SNP array verification indicated IBD
40	18+	FGR, previous adverse pregnancy	46, XY	6p21.31p11.1(33808094_58726706) x2 hmz, 6q11.1q16.1(61972918_93717031) x2 hmz	24.9 Mb 31.7 Mb	Parental SNP array verification indicated IBD
41	19	AMA	46, XX	6q14.3q21(87,299,268-110,741,585) x2 hmz	23.4 Mb	Parental SNP array verification indicated IBD
42	20	Thickened nuchal translucency (3.0 mm); AMA	46, XX	1q21.1q21.2(144,077,593-148,750,533) x2 hmz 3p21.31p21.1(48,166,782-53,172,233) x2 hmz 5q21.3q22.1(107,196,975-110,478,806) x2 hmz 12q21.31q21.33(82,446,525-91,707,400) x2 hmz 14q31.2q32.12(84,339,970-92,755,472) x2 hmz 15q24.1q25.3(73,065,223-87,467,262) x2 hmz 16p13.3(94,807-3,112,982) x2 hmz 17p12p11.2(15,838,698-22,170,994) x2 hmz	54.2 Mb (2%)	Parental SNP array verification indicated IBD

*The percentage is calculated by the sum of the size (Mb) of the LOH segments over 5 Mb divided by ~2781 Mb.

§Each case was classified based on its most important invasive diagnostic indication. The classification of indications, arranged in the order of importance are as follows: ultrasound anomalies, positive NIPT results, high risk for Down' screening, parental abnormal karyotype, consanguinity, previous adverse pregnancies, AMA, suspected fetal infection, and others.

AMA, advanced maternal age; AFI, amniotic fluid index; ARSA, aberrant left subclavian artery; BW, birth weight; BPD, biparietal diameter; FL, femur length; FGR fetal growth restriction; GW, gestational weeks; HC, head circumference; IBD, identity by descent; LOH, loss of heterozygosity; LP, likely pathogenic; Mat, maternal; MS-MLPA, methylation-specific multiplex ligation-dependent probe amplification; NIPT, noninvasive prenatal testing; OMIM, online mendelian

inheritance in man; Pat, paternal; P, pathogenic; PWS, prader-willi syndrome; RAA, right aortic arch; SNP, single nucleotide polymorphism; TOP, terminal of pregnancy; UPD, uniparental disomy; VSD, ventricular septal defect

Table 3 The detailed 64 prenatally diagnosed fetuses with LOH declined parental SNP array verification or further genetic testing

*The percentage is calculated by the sum of the size (Mb) of the LOH segments over 5 Mb divided by ~2781 Mb.

§Each case was classified based on its most important invasive diagnostic indication. The classification of indications, arranged in the order of importance are as follows: ultrasound anomalies, positive NIPT results, high risk for Down' screening, parental abnormal karyotype, consanguinity, previous adverse pregnancies, AMA, suspected fetal infection, and others.

AMA, advanced maternal age; BPD, biparietal diameter; dn, de novo; FGR, fetal growth restriction; hmz, homozygosity; NIPT, noninvasive prenatal testing; NT, nuchal translucency; RSA, recurrent spontaneous abortion; SNP, single nucleotide polymorphism; TOP, termination of pregnancy.

Figures

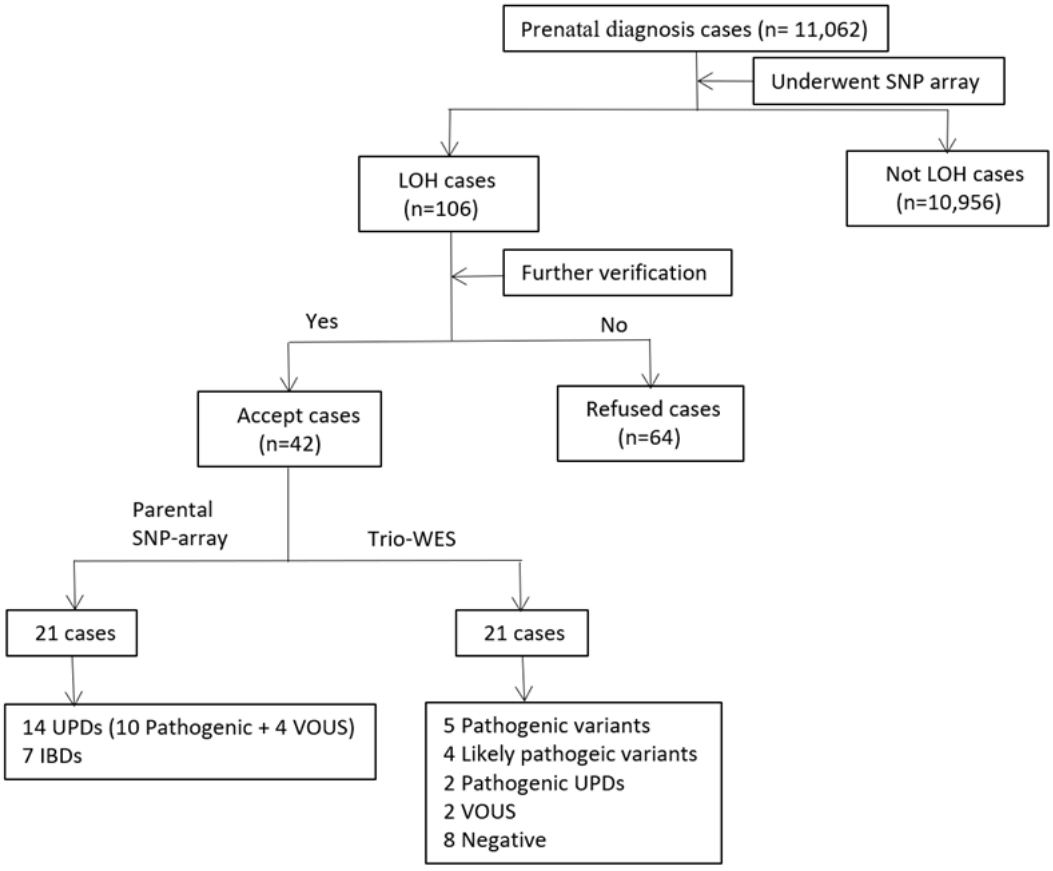


Figure 1

The flow chart of fetal LOH analysis in our cohort.

IBD, identity by descent; LOH, loss of heterozygosity; SNP array, single nucleotide polymorphism array; UPD, uniparental disomy; VOUS, variant of unknown significance; WES, whole exome sequencing.

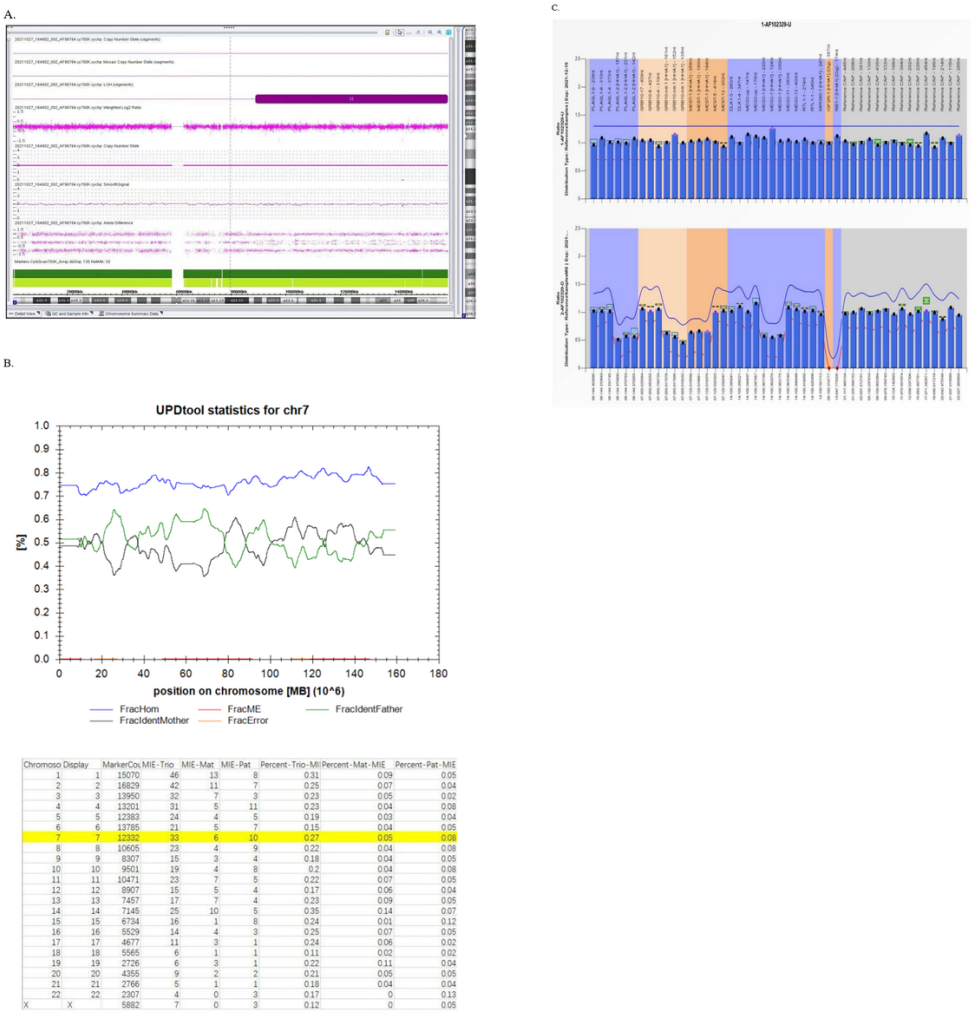
Case	GW	Prenatal imaging findings/Invasive testing indication	Fetal karyotype	Fetal LOH region detected by SNP-array results [hg19]	Size (Mb) /Percentage (%)*	Outcome
43	18+	Her husband carries 46, XY, t(4;5)(q31;q23), consanguineous marriage	46, XX, t (4;5) (q31; q23) pat	18p11.32p11.21(136,304-15,079,294) x2 hmz	15 Mb	Term birth, normal development
44	25	FGR, enhanced intestinal echo	46, XX	8p23.1p21.3(8117565_21609098) x2 hmz	13.5 Mb	Term birth, normal development
45	23+	Small femur length for gestation age; high risk of trisomy 22 detected by NIPT	46, XY	22q12.3q13.33(35,156,334-51,157,531) x2 hmz	16 Mb	Term birth, normal development
46	20	Del (8p) detected by NIPT; AMA	46, XY	8p23.3p23.1(168,483-6,999,220) x2 hmz, 8p23.1p12(8,117,564-32,069,805) x2 hmz	6.8 Mb 23.9 Mb	Term birth, normal development
47	18+	Consanguineous marriage; pregnant women had a child with mental retardation	46, XX	8p23.3p23.1(2,142,556-6,999,220) x2 hmz	67.9 Mb (3.63%)	Term birth Mental retardation
48	21	Enhanced intestinal echo; Pregnant women had three children with cardiac malformation	46, XX	13q14.2q22.3(49,157,476-78,073,267) x2 hmz	29 Mb	Term birth, normal development
49	12	Fetal cystic hygroma	46, XY	9q21.13q21.33(77,263,747-89,236,178) x2 hmz 9q22.33q31.1(101,366,537-107,749,439) x2 hmz 10q22.3q24.1(80,035,256-97,660,572) x2 hmz 14q31.1q32.2(79,435,542-97,071,550) x2 hmz 17q24.2q25.3(67,002,309-81,041,760) x2 hmz 18q22.3q23(71,555,375-77,997,606) x2 hmz 19q12q13.33(31,360,714-49,513,502) x2 hmz 21q21.1q22.13(19,573,963-39,057,622) x2 hmz	111.74 Mb (4.02%)	Missed abortion
50	14+	AMA	46, XY	9q22.33q33.1(101944435_121284276) x2 hmz	19.3 Mb	Missed abortion
51	20	Coarctation of the fetal aorta	46, XX	19p13.2p11(6939202_24462369) x2 hmz	17.5 Mb	Intrauterine demise
52	15	Hydrops fetalis	46, XY	5q11.1q35.3(49564628_180692321) x2 mos hmz (30%)	130 Mb	Missed abortion
53	16	Fetal giant bladder	46, XX	5q34q35.3(164130490_176629546) x2 hmz	12.5 Mb	Missed abortion
54	25	Fetal dysplasia or absence of left radius, abnormal left lower limb posture, single umbilical artery	46, XY	16q22.3q24.3(73294159_90146366) x2 hmz	16.8 Mb	TOP
55	15	Thickened NT (5 mm)	46, XX	3p14.2p11.1(59365038_90485635) x2 hmz 3q11.1q13.11(93558926_103243507) x2 hmz	31 Mb 10 Mb	Missed abortion
56	23	Small femur length and humerus length for gestation age	46, XX	4q28.3q31.3(133,718,289-154,569,367) x2 hmz	20.8 Mb	Term birth, normal development
57	26+	Fetal ventriculomegaly (1.4 cm), large for gestational age	46, XY	3q26.1q29(163,256,369-197,791,601) x2 hmz 5p13.1p11(41,029,137-46,313,469) x2 hmz 6q24.2q26(143,341,406-161,527,784) x2 hmz 12q13.2q21.2(56,011,100-77,134,151) x2 hmz 17q21.2q21.32(39,639,602-45,479,706) x2 hmz 21q21.3q22.2(28,124,165-42,352,287) x2 hmz	99.1Mb (3.57%)	TOP
58	24	Fetal infantile polycystic kidney disease?	46, XX	2q31.1q35(175,428,638-216,907,322) x2 hmz, 5q14.3q22.3(85,447,858-113,850,052) x2 hmz	41 Mb 28 Mb	TOP

59	28+	FGR, small fetal HC for gestation age	46, XY	5q15q22.2(94637916_112744413) x2 hmz	18.1 Mb	Preterm birth
60	19	AMA	46, XY	10q23.1q25.1(86,546,612-109,956,967) x2 hmz	23.4 Mb	Term birth, normal development
61	25	Fetal tetralogy of Fallot, thickened NT (4.7 mm)	46, XY	1p33p32.3(50,051,514-53,274,566) x2 hmz 2q23.3q24.1(153,771,280-158,783,675) x2 hmz 3q21.2q22.1(124,817,983-129,317,745) x2 hmz 3q22.1q23(133,262,566-139,418,898) x2 hmz 3q26.1q26.2(161,540,639-168,592,236) x2 hmz 7p22.3p21.2(2,707,568-13,857,235) x2 hmz 8q23.3q24.12(114,788,423-119,897,611) x2 hmz 9p22.1p13.3(19,696,747-36,125,149) x2 hmz 11q12.2q12.3(60,193,879-63,210,491) x2 hmz 11p11.2p11.12(45,781,075-51,550,787) x2 hmz 17q21.31q21.32(41,647,165-44,927,874) x2 hmz 17q25.1q25.3(71,965,953-75,785,426) x2 hmz 20p11.23p11.21(20,268,153-23,275,237) x2 hmz	77.5 Mb (2.8%)	TOP
62	23+	Amniocentesis: 46,XY,t(11;22) (q24.1;q12.3) dn; AMA	46, XY, t (11;22) (q24.1; q12.3) dn	3p13q13.31(71,435,373-116,447,779) x2 hmz	45.0 Mb	Term birth, normal development
63	22	Fetal diaphragmatic hernia, permanent right umbilical vein, high risk of trisomy 21 screening	46, XY	22q11.1q13.32(16,888,899-48,538,372) x2 hmz	31.6 Mb	TOP
64	19+	High risk of trisomy 21 screening, consanguineous marriage	46, XX	8q23.1q23.3(109,495,878-115,007,395) x2 hmz 8q24.21q24.3(128,517,572-143,689,390) x2 hmz 10q24.2q25.1(101,324,364-109,677,582) x2 hmz 11q24.2q25(123,916,776-132,360,867) x2 hmz 16q11.2q12.2(46,504,466-55,451,871) x2 hmz 19q13.12q13.32(36,345,999-45,532,009) x2 hmz	46.4 Mb (2.89%)	Term birth, normal development
65	18	Adverse pregnancy history	46, XY	3p26.1p24.1(8,494,626-26,413,121) x2 hmz	17.9 Mb	Term birth, normal development
66	18+	High risk of trisomy 21 screening	46, XX	4q32.1q34.3(161,662,054-181,126,952) x2 hmz	19.5 Mb	Term birth, normal development
67	19+	AMA, RSA	46, XY	15q21.1q24.2(49,174,353-76,415,329) x2 hmz	27.2 Mb	Term birth, normal development
68	30=	Fetal ventriculomegaly (1.4 cm)	46, XX	1q25.2q31.3(179,562,791-198,041,374) x2 hmz 1p33p32.3(49,189,774-53,588,443) x2 hmz 1p31.1p22.1(83,490,160-94,219,311) x2 hmz 3q22.2q22.3(135,242,870-138,329,862) x2 hmz 3p14.1p13(65,159,530-71,254,193) x2 hmz 3q12.1q12.3(99,386,363-102,630,046) x2 hmz 4q34.3q35.1(178,198,740-183,532,267) x2 hmz 5q33.3q34(157,473,329-162,124,378) x2 hmz 8p21.2p12(25,313,218-29,802,727) x2 hmz 9q22.1q31.2(90,844,062-108,221,369) x2 hmz 10q21.1q22.1(54,568,807-72,273,380) x2 hmz 11p11.2p11.12(45,959,522-51,550,787) x2 hmz 13q31.3q32.3(93,816,292-101,526,284) x2 hmz 18q11.1q11.2(18,552,516-23,353,126) x2 hmz 18p11.31p11.21(4,951,983-15,079,294) x2 hmz 20p13p12.3(61,794-5,436,062) x2 hmz	129.19 Mb (4.65%)	Term birth, hydrocephalus, abnormal fingers on both hands, learning disability
69	23	Fetal cleft palate	46, XY	8q24.22q24.3(134,714,740-146,292,734) x2 hmz	11.6 Mb	TOP
70	18+	AMA	46, XX	10q24.32q25.3(104,021,108-115,579,812) x2 hmz	11.5 Mb	Term birth, normal development
71	19+	AMA	46, XY	1p36.12p34.2(22,992,252-40,994,050) x2 hmz	18 Mb	Term birth, normal development

72	23+	Hydramnios, AMA	46, XX	2q31.1q32.2(177,156,393-190,026,211) x2 hmz	12.8 Mb	Preterm birth, normal development
73	20+	High risk of trisomy 21 screening	46, XX	15q14q21.1(37,475,111-48,299,651) x2 hmz	10.8 Mb	Term birth, normal development
74	22+	Hyperechoic nodules in fetal liver	46, XX	2q11.1q12.3(95,550,957-108,770,463) x2 hmz	13.2 Mb	Term birth, normal development
75	20	AMA, embryo arrest in one of the twin pregnancy	46, XY	5q11.1q13.2(49560858_68826246) x2 hmz 5p13.2p11(38119461_46383335) x2 hmz 10q11.22q21.1(48654362_59013629) x2 hmz 14q32.2q32.33(98824485_107285437) x2 hmz 17q11.1q11.2(25309336_30880382) x2 hmz 17p12p11.1(13895964_22217883) x2 hmz	19.26 Mb 8.26 Mb 10.36 Mb 8.46 Mb 5.57 Mb 8.32 Mb	Term birth, normal development
76	18+	AMA	46, XY	10q22.3q23.33(79424943_95211586) x2 hmz	15.79 Mb	Term birth, normal development
77	27	Small fetal BPD for gestation age, mild tricuspid regurgitation	46, XX	6q22.31q23.3(124043730_137024585) x2 hmz	12.98 Mb	Term birth, normal development
78	19	AMA	47, XX, +21	arr (21)x3 18p11.32p11.21(136,304-15,079,294) x2 hmz	14.9 Mb	TOP
79	21+	Thickened NT (4.7 mm)	46, XY	3p12.3p11.1(78796314_90485635) x2 hmz	11.7 Mb	Term birth, normal development
80	19+	Pregnant women with mental retardation, night blindness, consanguineous marriage	46, XY	13q13.3q21.33(38088920_71004437) x2 hmz	32.9 Mb	Term birth, normal development
81	25+	Fetal lung cystic adenoma		5q31.3q34(141922621_162299719) x2 hmz	20 Mb	TOP
82	22	Fetal enhanced intestinal echo	46, XX	1q32.2q44(208165416_245084139) x2 hmz	36.9 Mb	Term birth, normal development
83	18+	High risk of trisomy 1 and trisomy 8 detected by NIPT	46, XY	10q23.1q25.1(87343533_109730397) x2 hmz	22.4 Mb	Term birth, normal development
84	26	Fetal slightly thickened pulmonary valve, strephenopodia, pulmonary valve and tricuspid valve mild regurgitation	46, XY	3p26.2p25.1(2886527_13828221) x2 hmz 4p16.3p15.33(3473602_14373371) x2 hmz 5p13.3p11(31554333_46313469) x2 hmz	10.9 Mb 10.9 Mb 14.8 Mb	TOP
85	19	Absent fetal nasal bone, high risk of trisomy 18	46, XX	2p16.1p13.2(55018895_72337985) x2 hmz 2q11.2q31.1(101324333_177929684) x2 hmz 3q13.33q21.3(119592046_128125154) x2 hmz 4q34.3q35.2(181657468_190921709) x2 hmz 4q26q31.21(116436130_145124024) x2 hmz 5q23.3q33.3(128032159_157973399) x2 hmz 11q11q13.3(54827208_69837254) x2 hmz 11p14.3p11.12(21783630_51550787) x2 hmz 12q12q24.31(46091467_124915560) x2 hmz 15q22.31q24.1(66572692_73290903) x2 hmz 17p13.3p13.1(1365961_8388179) x2 hmz 17q22q24.1(55220296_64161582) x2 hmz 17q24.3q25.3(69423334_81041760) x2 hmz 21q22.12q22.3(36678533_48061211) x2 hmz	339.6 Mb (12.2%)	Term birth, normal development
86	18	hypoplastic nasal bone, high risk of trisomy 21 detected by NIPT	46, XY	3q12.1q13.2(99160747_111326722) x2 hmz	12.2 Mb	Term birth, normal development

87	18+	The husband of the pregnant woman carries 46,XY, t(2;6)(p21;q21)	46, XX	11p15.5p15.4(230751_5408252) x2 hmz	5.18 Mb	Term birth, normal development
88	30	FGR	46, XY	3q11.2q13.13(97766775_109823273) x2 hmz 4q27q32.1(121203882_156320512) x2 hmz	12 Mb 35 Mb	Preterm birth, normal development
89	19+	Deletion in 1p36.3 detected by NIPT, AMA	46, XX	1p36.33p36.13(888659_18328851) x2 hmz	17.4 Mb	Term birth
90	20	Thickened NT (3.2 mm)	46, XY	2q24.3q31.1(164542492_174491805) x2 hmz	10 Mb	Term birth, normal development
91	25+	Fetal portal-body venous shunt outside the liver	46, XY	4p15.2p11(22224153_49063479) x2 hmz	26.8 Mb	Term birth, normal development
92	19+	High risk of trisomy 21 screening	46, XX	8q13.3q21.13(72567811_83036411) x2 hmz	10.5 Mb	Term birth, normal development
93	18+	Deep notch a-wave of ductus venosus, adverse pregnancy history	46, XY	13q14.2q21.2(47320304_61602456) x2 hmz	14.3 Mb	Term birth, normal development
94	18	Adverse pregnancy history	46, XY	2q11.1q11.2(95550958_100799003) x2 hmz 9p24.1p13.2(8235898_36732597) x2 hmz 18q11.2q22.1(23795473_66441448) x2 hmz 20q13.12q13.32(43817586_56736674) x2 hmz	89.3 Mb (3.21%)	Term birth, normal development
95	18+	High risk of trisomy 21 screening	46, XY	18q12.2q21.1(35074699_45621145) x2 hmz	10.55 Mb	Term birth, normal development
96	26	Fetal right ventricle is slightly smaller than the left ventricle, slightly smaller the inner diameter of the pulmonary artery and left and right pulmonary artery, and mild tricuspid regurgitation, high risk of trisomy 21 screening	mos 47, XY, +22[3]/46, XY [58]	arr (22)x2.3 (30% mos) 22q12.3q13.33(36679058_51157531) x2 hmz	14.5 Mb	TOP
97	19+	High risk of fetal sex chromosome aneuploidy detected by NIPT, AMA	46, XX	3q13.13q22.2(110489883_134438659) x2 hmz	23.9 Mb	Term birth, normal development
98	20	Fetal bilateral choroid plexus cysts, intracardiac echogenic focus, high risk of fetal sex chromosome aneuploidy detected by NIPT	46, XY	3p13p11.1(72195690_90485635) x2 hmz 3q11.1q12.2(93558926_100589330) x2 hmz 4q31.21q32.3(145585354_165933062) x2 hmz 6q26q27(161702754_170896644) x2 hmz 11p13p11.12(36146925_51550787) x2 hmz 11q11q12.2(54827208_60193880) x2 hmz	75.6 Mb (2.72%)	Term birth, normal development
99	19+	The pregnant woman carries 46, XX, t(12;21)(q12;q22)	46, XX, t (12;21) (q12; q22.2) mat	3p12.3q12.2(78304909_100613493) x2 hmz	22 Mb	Term birth, normal development
100	20+	Fetal separation of right renal pelvis, intracardiac echogenic focus	46, XY	14q23.2q24.3(62101707_77219310) x2 hmz	15 Mb	Term birth, normal development
101	28	Fetal multiple calcification foci in fetal abdominal cavity	46, XY	2q24.1q24.3(156,461,811-165,665,567) x2 hmz 2p13.2p11.2(72,170,192-83,714,557) x2 hmz	9.2 Mb 20.7 Mb	Term birth, normal development
102	31	Fetal agenesis of the corpus callosum	46, XX	13q21.2q31.1(61,365,983-85,033,012) x2 hmz	23 Mb	TOP
103	25	FGR	46, XX	6q12q14.1(69812646_82725168) x2 hmz	12.9 Mb	Preterm birth, normal development
104	30+	FGR, amniocentesis: 47,XN,+mar[57]/46,XN[41]	47, XN, +mar [57]/46, XN [41] dn	6p12.3q13(47138118_74152240) x2.77 6p25.3p12.3(203877_47207081) x2 hmz 6q13q27(74056154_170896644) x2 hmz	 27.0 Mb 96.8 Mb	TOP
105	29+	FGR, increased intestinal echo	46, XY	12p13.2p12.1(10143600_22685434) x2 hmz	12.5 Mb	Term birth, normal development

106	21	AMA		46, X, inv(Y)(p11.2q11.2) mos 45%	arr (13)x2 mos hmz (45%)	Term birth, epilepsy, mild mental retardation



Chromoso	Display	MarkerCou	MIE-Trio	MIE-Mat	MIE-Pat	Percent-Trio-MI	Percent-Mat-MIE	Percent-Pat-MIE
1	1	15070	46	13	8	0.31	0.09	0.05
2	2	16829	42	11	7	0.25	0.07	0.04
3	3	13950	32	7	3	0.23	0.05	0.02
4	4	13201	31	5	11	0.23	0.04	0.08
5	5	12383	24	4	6	0.19	0.03	0.04
6	6	13785	21	5	7	0.15	0.04	0.05
7	7	12332	33	6	10	0.27	0.05	0.08
8	8	10605	23	4	9	0.22	0.04	0.08
9	9	8307	15	3	4	0.18	0.04	0.05
10	10	9501	19	4	8	0.2	0.04	0.08
11	11	10471	23	7	5	0.22	0.07	0.05
12	12	8907	15	5	4	0.17	0.06	0.04
13	13	7457	17	7	4	0.23	0.09	0.05
14	14	7145	25	10	5	0.35	0.14	0.07
15	15	6734	16	1	8	0.24	0.01	0.12
16	16	5529	14	4	3	0.25	0.07	0.05
17	17	4677	11	3	1	0.24	0.06	0.02
18	18	5565	6	1	1	0.11	0.02	0.02
19	19	2726	6	3	1	0.22	0.11	0.04
20	20	4355	9	2	2	0.21	0.05	0.05
21	21	2766	5	1	1	0.18	0.04	0.04
22	22	2307	4	0	3	0.17	0	0.13
X	X	5882	7	0	3	0.12	0	0.05

Figure 2

A 70.4 Mb loss of heterozygosity (LOH) was detected by SNP array (A), and further confirmed as maternal uniparental disomy 7 (UPD7) (B and C) for case 14.

Detection of a 70.4 Mb segmental LOH on 7q21.13q36.3 using a SNP array. Purple bars represent stretches of LOH occurring on 7q21.13q36.3. The allele difference panel indicates the genotype for each SNP.

Confirmation of low proportion mosaic maternal UPD7 using the methylation-specific multiplex ligation dependent probe amplification (MS-MLPA).

MS-MLPA revealed that the relative copy numbers of the three methylation probes in *MEST* gene (maternal methylation gene region, paternal methylation was preferentially expressed) were 0.66, 0.66 and 0.64, respectively, suggesting a possible low proportion mosaic UPD (7) mat. However, the experimental results are near the threshold range, so it cannot be interpreted accurately.

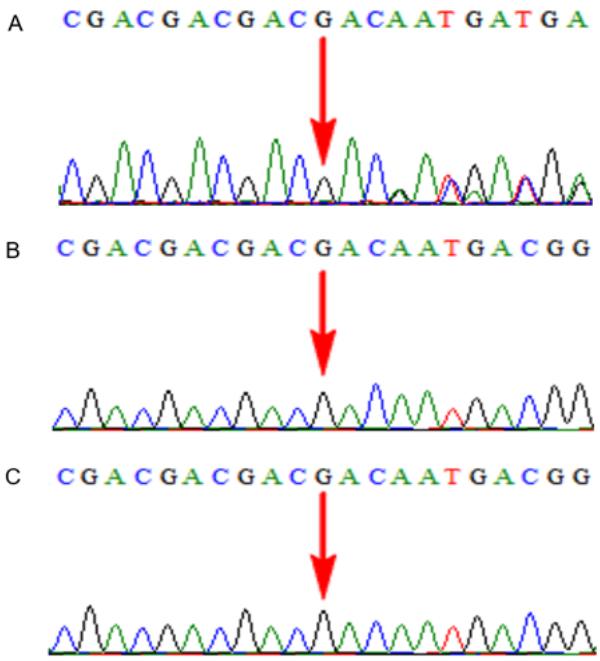


Figure 3

Trio whole exome sequencing results for case 15. The female infant harbored a de novo heterozygous mutation, NM _000095: c.1417_1419dup(p.D473dup), in *COMP* in the female infant.

A: The female infant; B: The mother; C: The father

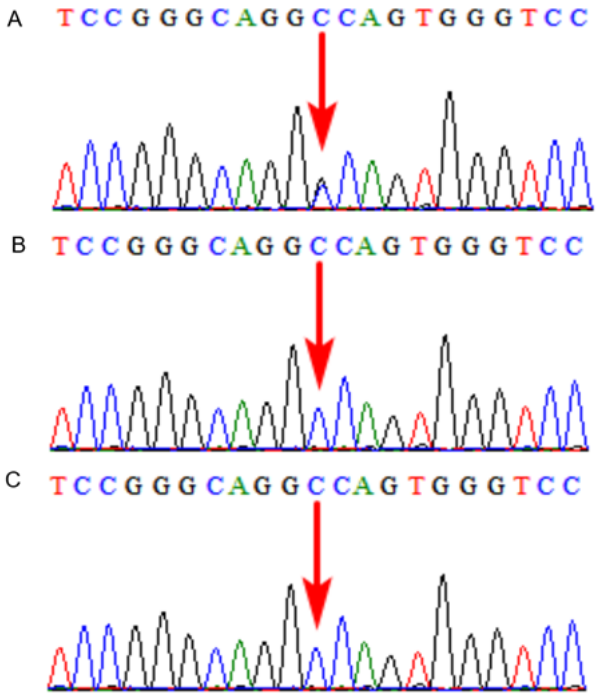


Figure 4

Trio whole exome sequencing results for case 18. The fetus harbored a de novo missense variant, NM_000088: c.1436G>C p.G479A, in *COL1A1* (120150) on chromosome 17.

A: The fetus; B: The mother; C: The father

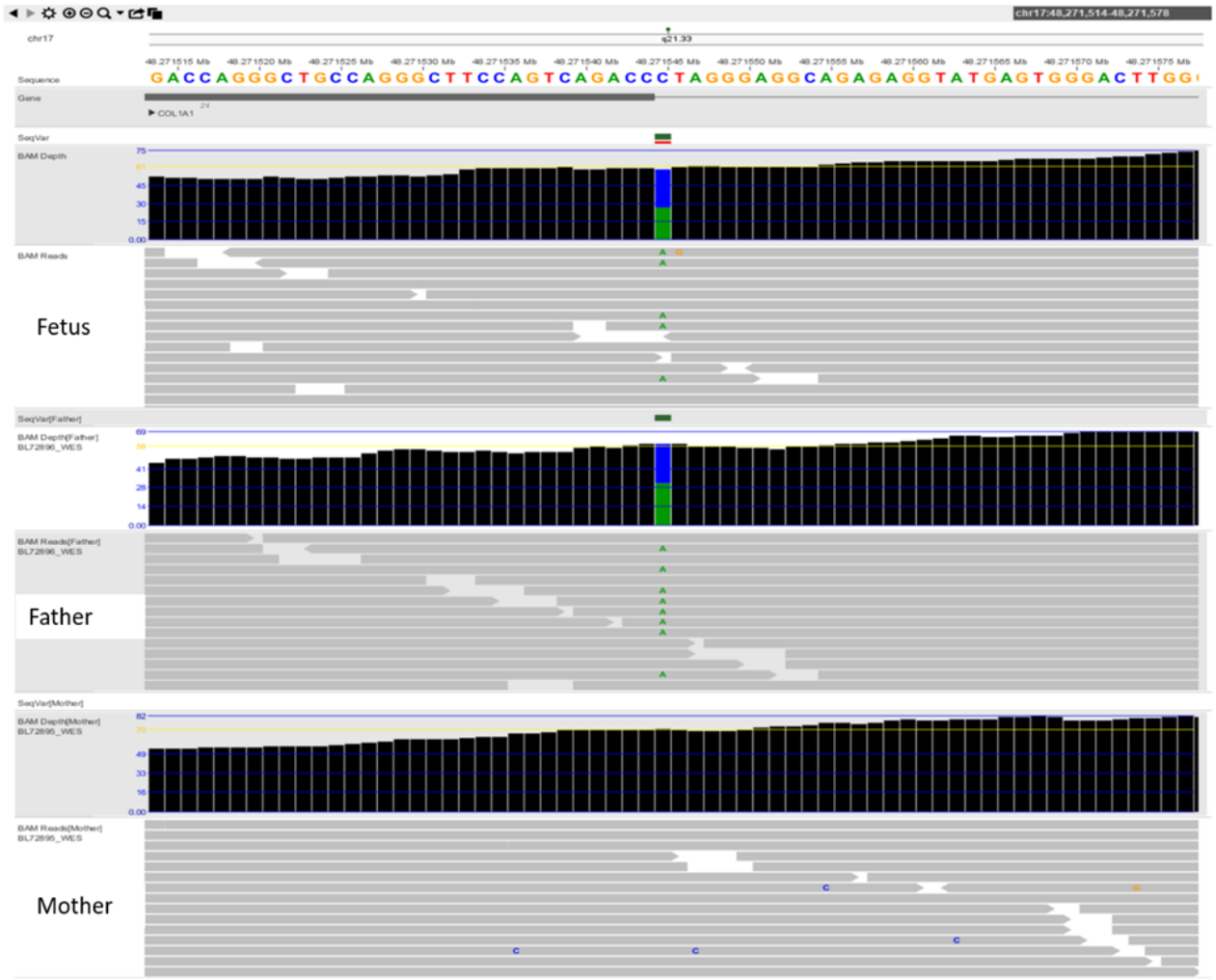


Figure 5

Trio whole-exome sequencing results for case 29. The fetus harbored an inherited paternally splicing variant, NM_000088.4: c.1615-1G>T (p. G802V), in *COL1A1* (120150) on chromosome 17.