Ultrasound Obstet Gynecol 2021; **57**: 314–323
Published online 7 January 2021 in Wiley Online Library (wileyonlinelibrary.com). **DOI:** 10.1002/uog.21951

Absence of heterozygosity detected by single-nucleotide polymorphism array in prenatal diagnosis

J. LIU, Z. HE, S. LIN, Y. WANG, L. HUANG, X. HUANG and Y. LUO

Department of Obstetrics & Gynecology, The First Affiliated Hospital of Sun Yat-Sen University, Guangzhou, People's Republic of China

KEYWORDS: absence of heterozygosity; chromosomal microarray analysis; CMA; genetic counseling; loss of heterozygosity; prenatal diagnosis; single-nucleotide polymorphism array; SNP; uniparental disomy

CONTRIBUTION

What are the novel findings of this work?
Absence of heterozygosity (AOH) most frequently involved chromosomes X, 2 and 16 on prenatal diagnosis, and the most common associated ultrasonographic abnormalities were multiple abnormalities, genitourinary malformations, skeletal malformations and small-for-gestational age.

What are the clinical implications of this work?
We propose a strategy for the management of AOH by further conducting serial ultrasound examinations, parental verification, whole-exome sequencing, placental study and effective follow-up.

ABSTRACT

Objectives *To investigate the general occurrence and clinical significance of absence of heterozygosity (AOH), detected by single-nucleotide polymorphism (SNP) array on prenatal diagnosis.*

Methods *We recruited pregnancies undergoing invasive prenatal diagnosis at our fetal medicine center over a 6-year period. All fetuses underwent SNP array using the Affymetrix CytoScan HD array platform. AOH was defined as a chromosomal homozygosity segment with neutral copy number. Cases with AOH over 10 Mb in size or with suspected pathogenicity were further analyzed, and the clinical features and outcome were reviewed.*

Results *Of 10 294 recruited fetuses, 100 (0.97%) with AOH were identified; in 81 (81.0%) of these, AOH occurred in a single chromosome, while 19 (19.0%) patients had multiple AOHs in different chromosomes. AOH was observed in all chromosomes, chromosomes X, 2 and 16 being the most frequently involved. The length of AOH ranged from partial chromosome (9.002–80.222 Mb) to the entire chromosome. Similar AOH regions displayed varied clinical manifestations. In total, 55 patients presented with concomitant ultrasound abnormalities, the most common being multiple abnormalities (14/55 (25.5%)), genitourinary malformations (8/55 (14.5%)), skeletal malformations (5/55 (9.1%)) and small-for-gestational age (5/55 (9.1%)). Notably, the rate of adverse perinatal outcome (including termination of pregnancy, neonatal death, fetal death, selective reduction and miscarriage) in fetuses with AOH and ultrasound abnormalities (30/48 (62.5%)) was higher than in those without ultrasound abnormalities (6/40 (15.0%)) ($P < 0.001$). Further non-invasive prenatal testing using cell-free fetal DNA from maternal blood indicated chromosomal copy number abnormalities in 11 patients; however, they were confirmed as AOH by SNP array of the amniotic fluid.*

Conclusions *Genetic counseling regarding a prenatal diagnosis of AOH remains challenging. To evaluate comprehensively its significance, we propose a management strategy involving further serial ultrasound examinations, parental verification, whole-exome sequencing, placental study and effective follow-up.* Copyright © 2019 ISUOG. *Published by John Wiley & Sons Ltd.*

INTRODUCTION

Currently, chromosomal microarray analysis (CMA) is the first-line diagnostic test for individuals with developmental disabilities or congenital anomalies[1]. In the field of prenatal diagnosis, CMA affords a 4.1–10% greater detection rate for chromosomal abnormalities than does conventional karyotyping in fetuses with an abnormality detected on ultrasound[2]. Single-nucleotide polymorphism (SNP) array is a common method

Correspondence to: Dr Y. Luo, Department of Obstetrics & Gynecology, The First Affiliated Hospital of Sun Yat-Sen University, 58 Zhong Shan Er Road, Guangzhou, 510080, People's Republic of China (e-mail: luoyanm@mail.sysu.edu.cn)

Accepted: 4 December 2019

Copyright © 2019 ISUOG. Published by John Wiley & Sons Ltd.

for CMA. Aside from chromosomal submicroscopic imbalances, a SNP array can additionally identify haploidy, triploidy and absence of heterozygosity (AOH) by analyzing SNP genotype information[3].

AOH, also known as loss of heterozygosity, refers to long contiguous stretches of homozygosity in a chromosome. Mechanisms leading to AOH include trisomy rescue, monosomy rescue, gamete complementation, post-fertilization errors and somatic recombination[4]. The pathogenesis of AOH includes homozygous mutation of recessive diseases and increased susceptibility to complex diseases[5,6], imprinting effects caused by uniparental disomy (UPD)[7], hidden mosaicism or confined placental mosaicisms (CPM)[8], and potential association with tumorigenesis[9]. When AOH in the imprinting regions is confirmed to have been inherited from only one parent, it can cause imprinting disorders such as Prader–Willi syndrome (maternal UPD15), Angelman syndrome (paternal UPD15), Silver–Russell syndrome (SRS, maternal UPD7) and Beckwith–Wiedemann syndrome (BWS, paternal UPD11)[10]. However, some AOH carriers present normal phenotypes, and the clinical significance of AOH is still unclear. Limited data regarding prenatally diagnosed AOH have been reported, rendering the interpretation of abnormal AOH and genetic counseling of patients difficult.

Owing to the variable manifestations and uncertain significance of AOH, better understanding of the correlation between AOH and its phenotype is important. We speculated that prenatal presentation, such as seen on ultrasound, may have implications for the further molecular diagnosis and prognosis of AOH. The aim of this study was therefore to investigate the general occurrence and clinical features of AOH diagnosed prenatally, and to explore the management strategy.

PATIENTS AND METHODS

Subjects

We reviewed pregnant women undergoing invasive prenatal diagnosis for all indications at the First Affiliated Hospital of Sun Yat-Sen University, China, from January 2013 to January 2019. Fetal samples were collected through chorionic villus sampling at 11 to 13 + 6 weeks' gestation, amniocentesis for extraction of amniotic fluid at 17 to 23 + 6 weeks or cordocentesis to obtain umbilical cord blood at or after 24 weeks, after obtaining informed consent from the parents. Gestational age was confirmed based on the last menstrual period and the ultrasound scan at 11 to 13 + 6 weeks.

In pregnancies with AOH, defined as a chromosomal homozygosity segment with neutral copy number, we collected basic information, ultrasonographic presentation, maternal serum screening results for aneuploidy, non-invasive prenatal testing (NIPT) results using cell-free fetal DNA in maternal plasma, results of prenatal diagnosis, pregnancy outcome and postnatal follow-up data. Pregnancy outcome was obtained from delivery records if the patient delivered in our hospital. Otherwise, the patients were followed up before and after delivery via telephone.

To obtain more information on the correlation between AOH results and phenotype, we additionally reviewed non-fetal patients who showed AOH in peripheral blood samples (tested through proband examination or parental verification) and cases in which AOH was detected in chorionic villi after miscarriage.

SNP array

Chorionic villi (15 mg), amniotic fluid (10 mL) or umbilical cord blood (1 mL) was collected, and genomic DNA was extracted using a QIAamp DNA Blood mini kit (Qiagen Inc., Hilden, Germany). Copy number variants (CNVs) and AOH were detected using a genome-wide high-resolution SNP array on a CytoScan HD (Affymetrix) platform containing 750 000 SNPs and 2 600 000 oligonucleotide probes. The raw data were analyzed using the ChAS3.0 software (Affymetrix). All procedures were performed following the manufacturers' protocols.

The location of the chromosome was described based on the genome version GRCH37/hg19. CNVs were classified according to the American College of Medical Genetics (ACMG) guidelines[11]. The laboratory reported microdeletions larger than 500 kb, microduplications larger than 1 Mb and AOHs with a fragment length over 10 Mb, unless the chromosomal aberration had certain or probable pathogenicity. It is believed that 10 Mb is a reasonable cut-off level to report an incidental AOH in populations[4]. After obtaining informed consent, parental blood samples were also collected to verify the source of fetal AOH.

Karyotyping and whole-exome sequencing

Samples were cultured and prepared for Giemsa banding according to a standard cytogenetic protocol. Karyotype analysis and description were based on the International System for Human Cytogenetic Nomenclature (2013)[12]. To detect potential recessive pathogenic genes, whole-exome sequencing (WES) was further performed after obtaining parental consent. Sample DNA was extracted, fragmented randomly, purified and enriched to construct DNA libraries. The DNA libraries were sequenced using the NextSeq500 sequencer according to the manufacturer's protocols (Illumina, San Diego, CA, USA). Burrows–Wheeler Aligner software (version 0.59) was used to compare the sequences with the reference sequence of GRCh37.p10[13]. Gene mutation variants were interpreted according to ACMG guidelines (2015)[14].

Databases

The significance of CNV, AOH and gene-mutation results was interpreted by referring to online databases, including PubMed (http://www.ncbi.nlm.nih.gov/

Copyright © 2019 ISUOG. Published by John Wiley & Sons Ltd.

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), Cases with 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/).

The study was approved by the Ethics Committee of the First Affiliated Hospital of Sun Yat-Sen University (ID: [2019]285). Informed consent was signed by all parents before undergoing invasive prenatal diagnosis.

Statistical analysis

The χ^2 test, Fisher's exact test and Student's *t*-test were applied to analyze the data, using SPSS 22.0 software (SPSS Inc., Chicago, IL, USA); $P < 0.05$ was considered to indicate statistical significance.

RESULTS

Patient characteristics

In all 10 294 fetuses undergoing invasive prenatal diagnosis during the 6-year study period, the SNP array was performed successfully, and a total of 100 fetuses (0.97%) with AOH were detected, of which 98 (0.95%) had AOH > 10 Mb and two (Cases 55 and 57) had AOH ≤ 10 Mb with suspected pathogenicity. Of the fetuses with AOH in the cohort, 39 were from nulliparous women and 61 were from parous women. There were 85 fetuses from singleton pregnancies and 15 from twin pregnancies. Mean maternal age was 32 ± 5 (range, 19–43) years. Of the 100 fetuses, 89 had been conceived naturally and 11 had been conceived through assisted reproductive technology (ART). The overall rate of ART in the 10 294 fetuses was 7.7% (796/10 294), while it was 11.0% (11/100) in the AOH group, with no statistically significant difference ($P > 0.05$). Mean gestational age at invasive prenatal diagnosis in all pregnancies was 21 ± 6 (range, 11 + 2 to 40) weeks, and the corresponding mean gestational age in the AOH group was 23 ± 5 (range, 11 + 5 to 31 + 5) weeks, with no statistically significant difference ($P > 0.05$). The indications for invasive prenatal diagnosis in the 100 patients with AOH are listed in Table 1. Among the six cases from consanguineous couples (Cases 91, 94, 96, 97, 98 and 100), two of the four parous women had had a previous adverse pregnancy with a recessive disorder. Data regarding detailed CMA results, ultrasound manifestations and outcomes in each of the 100 prenatally diagnosed cases are provided in Table S1.

In addition, 11 cases of AOH were detected in chorionic villous tissue following miscarriage (Table S2) and 35 non-fetal AOH cases (age range, 6 days to 43 years) were detected in peripheral blood samples (Table S3).

Table 1 Indications for invasive prenatal diagnosis in 10 294 fetuses, overall and in those with absence of heterozygosity (AOH)

Indication	*All fetuses (n = 10 294)*	*Fetuses with AOH (n = 100)*
Ultrasound abnormality*	6161 (59.85)	55 (55)
High risk on maternal serum screening	1502 (14.59)	12 (12)
Parental genetic factors	797 (7.74)	2 (2)
Abnormal karyotype†	451 (4.38)	1 (1)
Gene mutation of monogenic disease	318 (3.09)	1 (1)
Microdeletion or microduplication	15 (0.15)	0 (0)
Abnormal family history‡	13 (0.13)	0 (0)
Advanced maternal age	706 (6.86)	2 (2)
Previous adverse pregnancy	583 (5.66)	13 (13)
Abnormal NIPT result	319 (3.10)	8 (8)
Suspected intrauterine infection	108 (1.05)	0 (0)
Consanguineous marriage	6 (0.06)	4 (4)
Other§	112 (1.09)	4 (4)

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 abnormalities, abnormal non-invasive prenatal testing (NIPT) results, high risk on maternal serum screening, parental genetic factors, consanguineous marriage, previous adverse pregnancy, advanced maternal age, suspected intrauterine infection and other. *Ultrasound abnormalities included structural malformations, soft-marker anomalies, small-for-gestational age or fetal overgrowth, amniotic fluid volume abnormality and abnormal blood flow on Doppler ultrasonography. †Abnormal karyotype included chromosomal balanced translocation, inversion, duplication and numerical chromosomal abnormalities. ‡Abnormal family history referred to parent with intellectual disability, ataxia or epilepsy. §Other indications included medication use or toxin exposure during pregnancy, fetal abnormal karyotype and suspected maternal–fetal blood group incompatibility.

General occurrence of AOH and chromosomes with high incidence of AOH

The prenatal detection rate of AOH overall was 0.97% (100/10 294), and the rate of AOH > 10 Mb was 0.95% (98/10 294). In 81 (81.0%) patients with AOH it occurred in a single chromosome, while in 19 (19.0%) patients, multiple AOHs were detected in different chromosomes. Of the 19 patients with multiple AOHs, six were from consanguineous marriages and in the other 13 there was no indication of any possible consanguinity of the parents. Although AOH was detected in all chromosomes, the distribution was not random. When AOH occurred in a single chromosome, the most frequently involved chromosomes were chromosomes X (12/81 (14.8%)), 2 (10/81 (12.3%)) and 16 (7/81 (8.6%)), followed by chromosomes 18, 9, 1, 7, 8 and 12 (Figure 1, Table S1). AOH occurred throughout the chromosome in 10 patients and covered almost the entire chromosome in three patients; the affected chromosomes were chromosomes 1, 2, 5, 7, 9, 13, 16, 18, 19 and X. In other cases, AOH occurred in a partial fragment of the chromosome, ranging from 9.002 to 80.222 Mb. In total, four patients with

Copyright © 2019 ISUOG. Published by John Wiley & Sons Ltd.

mosaic AOH were detected, involving the whole length of chromosome 2, multiple fragments in chromosome 2, 6q16.3q27 and 18q11.1q23, respectively. The proportion of mosaicism ranged from 10% to 50%.

The 11 cases of AOH detected in chorionic villus samples following miscarriage included eight cases with isolated AOH (six in chromosome X, one in chromosome 18 and one in chromosome 3), two cases with a combination of aneuploidy and AOH, and one case with multiple AOHs (Table S2).

Of the 35 non-fetal AOH cases, 24 had AOH in a single chromosome, mostly involving chromosome 7 (5/24 (20.8%)) or X (3/24 (12.5%)), followed by chromosomes 2, 5, 6, 15 and 16, while 11 cases had multiple AOHs in different chromosomes (Table S3).

In prenatally diagnosed cases, in chromosome X, the regions with a high incidence of AOH were Xp11.23p11.1 and Xq13.1q21.1; however, the prenatal presentations and outcomes of cases with AOH in the same region varied, including multiple ultrasound abnormalities, preterm birth, term birth and termination of pregnancy (TOP) (Table S1).

Isolated AOH in chromosome 2 was detected prenatally in 10 patients, of which four presented with ultrasound abnormalities, including small-for-gestational age (SGA), defined as estimated fetal weight $< 10^{th}$ percentile, hydronephrosis, absence of nasal bone, hypospadias, ventriculomegaly, polyhydramnios or oligohydramnios, resulting in TOP ($n = 3$) or neonatal death ($n = 1$). The other six patients showed no obvious abnormal phenotype on prenatal ultrasound, and had a favorable outcome. Among the non-fetal cases, in a premature infant (Case 112) diagnosed with hypotonia and intellectual disability, SNP array indicated paternal UPD2, with normal findings on karyotyping and WES.

For AOH in chromosome 16, the length ranged from 9.002 Mb to the whole length, and AOH was distributed in all regions of chromosome 16. Six out of seven fetuses with AOH in chromosome 16 had abnormal ultrasound findings, which included SGA, ascites, intra-abdominal

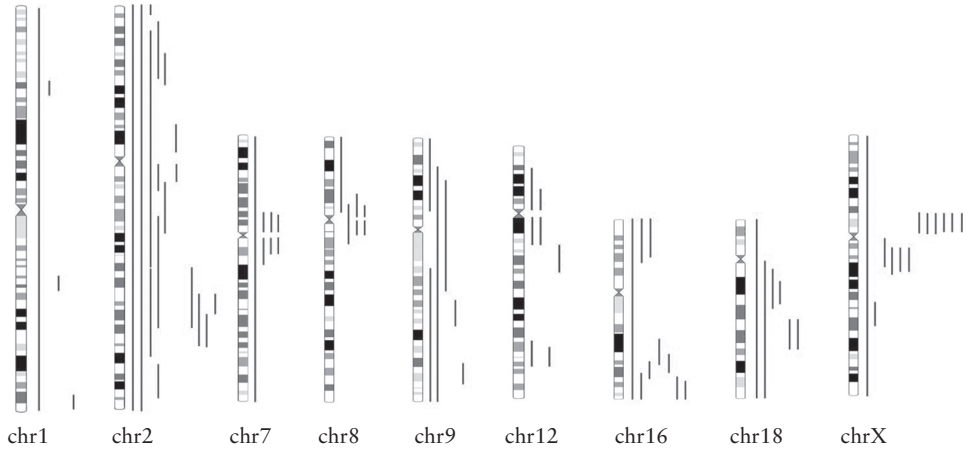


Figure 1 Distribution of high-incidence regions of absence of heterozygosity (AOH) greater than 10 Mb. Lines to right of chromosome represent location of AOH carried by each patient. Length of lines shows range of corresponding AOH region. AOH most frequently involved chromosomes X, 2 and 16, followed by chromosomes 18, 9, 1, 7, 8 and 12.

Table 2 Classification of abnormal ultrasound findings in 55 fetuses diagnosed prenatally with absence of heterozygosity

Ultrasound finding	*Detailed manifestation*	n *(%)*
Multiple malformations		14 (25.5)
Isolated structural malformation		23 (41.8)
Genitourinary system	Renal effusion, hypospadias, polycystic kidney, renal enlargement, increased renal echogenicity, separation of renal pelvis, renal dysplasia, bladder exstrophy	8 (14.5)
Skeletal system	Femoral fracture, absent fibula, suspected abnormal number of ribs, abnormal posture of right foot	5 (9.1)
Central nervous system	Exencephaly, pachygyria, cerebellar dysplasia, subependymal cyst, Blake's pouch cyst	3 (5.5)
Digestive system	Enhanced liver echo, enlarged gallbladder, intestinal dilatation	3 (5.5)
Cardiovascular system	Ventricular septal defect, atrial septal defect, aortic stenosis, descending aorta curving, cardiac macrovascular variations	2 (3.6)
Fetal hydrops	Hydroderma, pleural effusion, ascites	2 (3.6)
Isolated ultrasound soft-marker anomaly	Thickened nuchal translucency or nuchal fold*, nasal bone absence, intracardiac echogenic focus	7 (12.7)
Isolated SGA	—	5 (9.1)
Other abnormal findings	Polyhydramnios, oligohydramnios, absence of a-wave of ductus venosus	6 (10.9)

*Thickened nuchal translucency defined as > 3.0 mm; thickened nuchal fold defined as > 6.0 mm. SGA, small-for-gestational age.

Copyright © 2019 ISUOG. Published by John Wiley & Sons Ltd.

Table 3 Ultrasound findings, results of genetic testing and outcome in 14 fetuses diagnosed prenatally with absence of heterozygosity (AOH) that showed multiple ultrasound abnormalities

Case	*GA (weeks)*	*Ultrasound findings*	*Karyotype*	*SNP array results*	*Size (Mb)*	*Outcome*
5	30 + 4	SGA, hypospadias, small nasal bone	46,XY	2p25.3q37.3(15702-242775901) × 2 hmz	242.76	TOP
6	29 + 1	SGA, short nasal bone, cerebral ventriculomegaly	46,XX	Multiple large fragment AOH in chromosome 2	132.64	Died 1 h after preterm birth at 33 + 3 weeks
14	30 + 0	SGA, absence of gallbladder, oligohydramnios	46,XY	Multiple large fragment AOH in chromosome 2	Mosaicism 10–30%	TOP
24	13 + 5	Hydroderma, megabladder	46,XY	7p12.3p11.1(47743255-58019983) × 2 hmz 7q11.21q11.23(62461703-73446436) × 2 hmz	10.277 10.985	Intrauterine death
42	26 + 0	Persistent left superior vena cava, cerebral ventriculomegaly	46,XY	12q13.13q14.3(54102901-66 147 024) × 2 hmz Yq11.223q11.23 (24876522-28112869) × 2 dup	12.044 3.236	Term birth, BW 2.75 kg
46	25 + 2	Excessive growth of abdominal circumference, ascites, polyhydramnios	46,XY	14q32.11q32.33(91826842-107285437) × 2 hmz Xp22.33(859808-2 859 117) × 3 dup	15.46 2.00	TOP at 25 + 3 weeks, fetal weight 1.3 kg
49	22 + 0	SGA, ascites, lemon head, increased RI of umbilical artery, increased MCA-PSV	46,XX	15q14q25.3(36248978-88131599) × 2 hmz (paternal UPD 15)	51.88	TOP at 28 weeks, fetal weight 0.814 kg
53	24 + 1	Ascites, intra-abdominal calcification, cardiomegaly, thickened nuchal fold*	46,XY	16q21q22.3(60136253-73345707) × 2 hmz	13.21	TOP at 29 weeks
57	22 + 0	SGA, short long bones, left superior vena cava	46,XX	16q23.2q24.3(81161763-90163275) × 2 hmz	9.002	TOP at 33 weeks, fetal weight 1.51 kg
66	25 + 0	Mixed mass in mouth and face, polyhydramnios	46,XX	18q11.1q23(18310606-78077248) × 2 hmz, mosaicism	59.76	Lost to follow-up
72	30 + 5	Absent stomach, polyhydramnios, thickened nuchal fold*, single umbilical artery	46,XX	Xp11.23p11.11(47108065-58337890) × 2 hmz	11.23	TOP
73	30 + 3	SGA, absent nasal bone, absent 12th rib, increased RI of umbilical artery	46,XX	Xp11.23p11.1(47277730-58337890) × 2 hmz 10q11.22(47059391-48006310) × 1 del	11.06 0.947	Preterm birth at 35 weeks, BW 1.38 kg, intestinal dysplasia
87	22 + 3	Polydactyly, cerebral ventriculomegaly, renal effusion	46,XX	Multiple AOH in chromosomes 1, 2, 3, 4, 6, 8, 9, 11, 17, 21	212.779	Lost to follow-up
89	24 + 2	Right aortic arch, aberrant left subclavian artery, clubfoot	46,XX	Multiple AOH in chromosomes 1, 6, 8, 22	44.33	Term birth, BW 3.54 kg

As all single-nucleotide polymorphism (SNP) array analyses were run in array form (arr) with Human Genome build 19 (hg19), notation 'arr[hg19]' has been removed from SNP array results. *Thickened nuchal fold defined as > 6.0 mm. BW, birth weight; del, deletion; dup, duplication; GA, gestational age at which ultrasound abnormalities were initially screened; hmz, homozygous; MCA-PSV, peak systolic velocity of middle cerebral artery; RI, resistance index; SGA, small-for-gestational age; TOP, termination of pregnancy; UPD, uniparental disomy.

calcification, cardiomegaly, persistent left superior vena cava, short long bones, cardiac macrovascular variations and thickened nuchal fold (> 6.0 mm). The subsequent pregnancy outcomes were TOP ($n = 3$), selective reduction of a twin pregnancy ($n = 1$), intrauterine death ($n = 1$) and loss to follow-up ($n = 1$). In the other fetus with AOH in chromosome 16 (Case 58), UPD16 was detected without an abnormal prenatal phenotype, and it was born at term.

Ultrasound presentation in fetuses with AOH

The distributions of the ultrasound presentations are shown in Table 2. In total, 55 fetuses showed concomitant abnormalities on ultrasound screening, including 37 (67.3%) with a structural abnormality, 7 (12.7%) with an abnormal soft marker, 5 (9.1%) with isolated SGA and 6 (10.9%) with other findings. The most common ultrasonographic structural abnormalities were multiple malformations (14/55 (25.5%)) (Tables 2 and 3), genitourinary malformations (8/55 (14.5%)) and skeletal-system malformations (5/55 (9.1%)). In addition, malformations of the nervous, digestive and cardiovascular systems and fetal hydrops were detected. Soft-marker anomalies included thickened nuchal translucency (> 3.0 mm) or nuchal fold in four cases, absent nasal bone in two cases and intracardiac echogenic focus in one case.

Copyright © 2019 ISUOG. Published by John Wiley & Sons Ltd.

Table 4 Additional ultrasound findings, results of genetic testing and outcome in 11 fetuses diagnosed prenatally with absence of heterozygosity (AOH) that were small-for-gestational age (SGA)

Case	*GA (weeks)*	*Additional ultrasound findings*	*Karyotype*	*SNP array results*	*Size (Mb)*	*Outcome*
5*	30 + 4	Hypospadias, small nasal bone	46,XY	2p25.3q37.3 (15702-242775901) × 2 hmz	242.76	TOP
6*	29 + 1	Short nasal bone, cerebral ventriculomegaly	46,XX	Multiple large fragment AOH in chromosome 2	132.64	Died 1 h after preterm birth at 33 + 3 weeks
14*	30 + 0	Absence of gallbladder, oligohydramnios	46,XY	Multiple large fragment AOH in chromosome 2	Mosaicism 10–30%	TOP
29†	30 + 0	—	46,XY	8p12p11.1(29153825-43778914) × 2 hmz	14.625	Term birth, neonatal death
				8q11.1q11.23(46913605-53808648) × 2 hmz	6.859	
30†	30 + 0	—	46,XY	8p11.23p11.1(37222081-43778914) × 2 hmz	6.56	Term birth, neonatal death
				8q11.1q11.23(46913605-53808648) × 2 hmz	6.90	
47	24 + 4	—	46,XX	14q11.2q23.1(21108964-58455758) × 2 hmz	37.35	Term birth
49*	22 + 0	Ascites, lemon head, increased RI of umbilical artery, increased MCA-PSV	46,XX	15q14q25.3(36248978-88131599) × 2 hmz (paternal UPD 15)	51.88	TOP at 28 weeks, fetal weight 0.814 kg
52	26 + 0	—	Unknown	16p13.3p12.2(89560-21620447) × 2 hmz	21.53	Intrauterine death at 26 weeks
				16q23.1q24.3(77916190-90163275) × 2 hmz	12.25	
56	17 + 0	SGA in one fetus of dichorionic diamniotic twin pregnancy, increased RI of umbilical artery	46,XY	16p13.3(89560-7705423) × 2 hmz	7.616	Selective reduction
				16p13.3p12.3(7717327-19208505) × 2 hmz	11.491	
				16q22.2q23.2(71157422-80126717) × 2 hmz	8.969	
57*	22 + 0	Short long bones, left superior vena cava	46,XX	16q23.2q24.3(81161763-90163275) × 2 hmz	9.002	TOP at 33 weeks, fetal weight 1.51 kg
73*	30 + 3	Absent nasal bone, absent 12th rib, increased RI of umbilical artery	46,XX	Xp11.23p11.1(47277730-58337890) × 2 hmz	11.06	Preterm birth at 35 weeks, BW 1.38 kg, intestinal dysplasia
				10q11.22(47059391-48006310) × 1 del	0.947	

As all single-nucleotide polymorphism (SNP) array analyses were run in array form (arr) with Human Genome build 19 (hg19), notation 'arr[hg19]' has been removed from SNP array results. *Cases 5, 6, 14, 49, 57 and 73 are also listed in Table 3. †Cases 29 and 30 were twins from a dichorionic diamniotic twin pregnancy. BW, birth weight; del, deletion; GA, gestational age at which SGA was initially screened; hmz, homozygous; MCA-PSV, peak systolic velocity of middle cerebral artery; RI, resistance index; TOP, termination of pregnancy; UPD, uniparental disomy.

In the group with ultrasound abnormalities, AOH mostly involved chromosomes X, 16, 18, 2 and 12, whereas AOH mostly occurred in chromosomes X and 2 in the group without ultrasound abnormalities. On comparing those with and those without ultrasound abnormalities, we found that all fetuses with AOH in chromosomes 5, 6, 12, 17, 18 and 22 had ultrasound abnormalities, but the outcomes varied from term birth to intrauterine death.

SGA was detected prenatally in 11 (11.0%) fetuses (Table 4), of which five had SGA as an isolated ultrasound finding. The outcomes of these 11 fetuses included TOP ($n = 4$), neonatal death ($n = 3$), fetal death ($n = 1$), selective reduction ($n = 1$), preterm birth ($n = 2$, of which one resulted in neonatal death) and term birth ($n = 3$, of which two resulted in neonatal death). The incidence of fetal or neonatal death was significantly higher in fetuses with AOH and SGA than in those without SGA (36.4% (4/11) *vs* 4.5% (4/89), respectively). Among the 35 non-fetal AOH cases, eight children showed growth delay, of whom three were SGA at birth. The most frequently involved chromosomes were chromosomes 16 ($n = 3$) and 2 ($n = 3$) in prenatal cases with SGA, and chromosome 7 in non-fetal cases with growth delay ($n = 3$).

Correlation between aberrant NIPT results and AOH

NIPT was conducted according to maternal choice using cell-free fetal DNA from maternal blood. A total of 319 fetuses showed chromosomal copy number abnormalities, including trisomy, deletion and duplication. Among fetuses with a positive NIPT result, 11 showed AOH on a SNP array; in nine of these fetuses, AOH was confirmed in the chromosome that showed aberrant NIPT results, whereas two fetuses showed AOH in a different chromosome that did not yield a positive NIPT result (Table 5).

Copyright © 2019 ISUOG. Published by John Wiley & Sons Ltd.

Table 5 Ultrasound findings, results of genetic testing and outcome in 11 fetuses diagnosed prenatally with absence of heterozygosity on single-nucleotide polymorphism (SNP) array that had discordant non-invasive prenatal testing (NIPT) results

Case	*GA (weeks)*	*Ultrasound findings*	*NIPT result*	*Karyotype*	*SNP array results*	*Size (Mb)*	*Outcome*
18	19 + 0	—	Dup in chr 4	46,XX	4p16.3p15.1(46690-32732470) × 2 hmz	32.686	Term birth, BW 3.5 kg
					4q22.3q31.1(95254574-140896353) × 2 hmz	45.642	
					4q34.2q35.2(177272003-190921709) × 2 hmz	13.65	
23	17 + 6	—	Dup in chr 7	46,XX	7p22.3q36.3 (44166-159119220) × 2 hmz pat	159.1	Preterm birth at 34 weeks, BW 2.45 kg
27	19 + 4	—	Del in chr 8	46,XY	8p23.3p11.21(168483-42150135) × 2 hmz	41.98	Lost to follow-up
31	19 + 3	—	Dup in chr 9	46,XX	9p24.3p13.1(192128-40087758) × 2 hmz	39.896	Term birth
					9q21.11q34.3(71013799-141025328) × 2 hmz	70.012	
32	19 + 5	Enhanced liver echo	T21 high risk, dup in chr 9	47,XX + 21	9p22.3q34.3(15339721-141025328) × 2 hmz	94.76	TOP, fetal weight 0.518 kg
					21q11.2q22.3(15190686-48097372) × 3 dup	32.91	
37	19 + 2	—	Dup in chr 11	46,XX	11q12.2q25(60738201-134 942 626) × 2 hmz	74.20	Lost to follow-up
					11q12.2(61003213-61 237 552) × 4 dup	0.234	
43	21 + 6	—	Del in chr 13	46,XX	13q13.2q34(34873883-115095705) × 2 hmz	80.222	Lost to follow-up
54	25 + 0	Descending aorta curvature, cardiac macrovascular variations	Dup in chr 16	46,XX	16q23.2q24.3(79773290-90 163 275) × 2 hmz	10.39	TOP at 30 weeks
					16q12.2(53763731-53 909 306) × 1 del	0.146	
61	18 + 0	Thickened nuchal translucency*	T18 high risk	46,XY	(18) × 2 hmz		Lost to follow-up
74	29 + 5	—	T13 high risk	46,XX	Xp11.23p11(46568519-58337890) × 2 hmz	11.769	Preterm birth at 36 + 4 weeks, BW 2.7 kg
75	20 + 0	—	Dup in chr 16	46,XX	Xp11.23p11.21(46627554-57090151) × 2 hmz	10.463	Preterm birth at 36 weeks, BW 1.96 kg

As all SNP array analyses were run in array form (arr) with Human Genome build 19 (hg19), notation 'arr[hg19]' has been removed from SNP array results. *Thickened nuchal translucency defined as > 3.0 mm. BW, birth weight; chr, chromosome; Del/del, deletion; Dup/dup, duplication; GA, gestational age at invasive prenatal diagnosis; hmz, homozygous; pat, paternal; T, trisomy; TOP, termination of pregnancy.

Relationship between AOH and UPD

AOH that covered the entire length of the chromosome was considered as complete UPD, whereas AOH that covered nearly the entire length was considered as complete or segmental UPD. To confirm the source of AOH involving the partial chromosome, 21 cases of AOH were verified by parental blood samples, of which two (9.5%) had UPD (maternal UPD15 and maternal UPD16).

Notably, in Case 57, amniocentesis was performed in a 43-year-old pregnant woman, as ultrasound indicated a fetus with persistent left superior vena cava and short long bones. AOH with a size of 9.002 Mb was observed in 16q23.2q24.3 by SNP array. Trio WES and SNP analysis were performed in fetal and parental samples; the abnormal chromosomal variation was found to be derived from maternal UPD16, and it contained a 78.32-Mb heterodisomy and an 8.83-Mb isodisomy. As the pregnancy progressed, the fetus developed severe fetal growth restriction, and the parents decided to terminate the pregnancy at 33 weeks, at which point the fetus weighed 1.51 kg.

Karyotyping and gene mutation results

Karyotyping was performed successfully in 94 out of 100 fetuses with AOH. Of the remaining six patients, five underwent only SNP array in our center, while one patient had a failed culture. In total, 90 cases yielded normal results, and abnormal results included 47,XX+21 ($n = 2$), 47,XXY and 48,XX,+2mar (inherited from the father). WES was performed in four cases to detect gene mutation, and two results were significant. Femoral fractures were detected by ultrasound screening at 29 weeks in a pair of monochorionic diamniotic twins (Cases 59 and 60). SNP array detected a 19.56-Mb AOH in 17q12q22 in Case 59 and a 19.58-Mb AOH in 17q12q23 in Case 60. WES showed that the fetuses carried a homozygous

Copyright © 2019 ISUOG. Published by John Wiley & Sons Ltd.

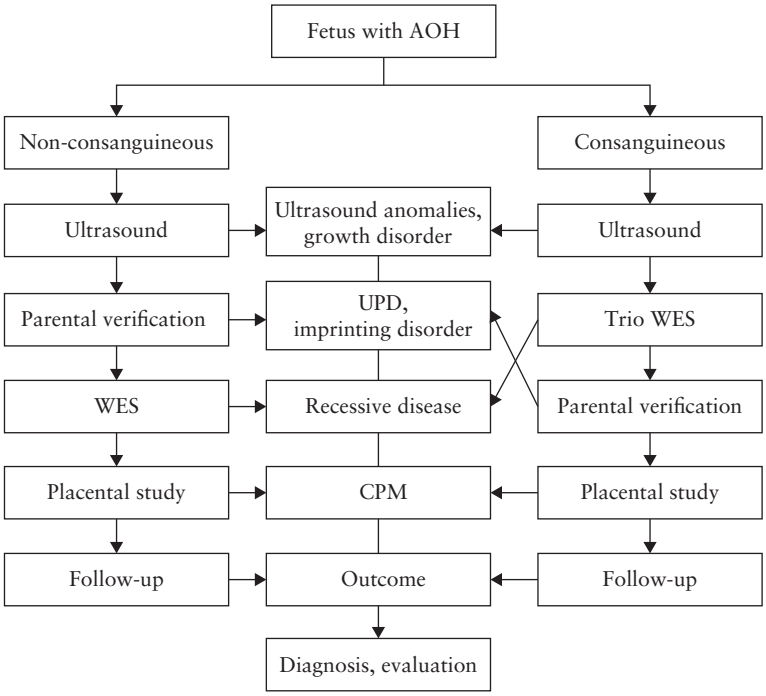


Figure 2 Strategy suggested to manage fetuses with absence of heterozygosity (AOH) detected by single-nucleotide polymorphism array. CPM, confined placental mosaicisms; UPD, uniparental disomy; WES, whole-exome sequencing.

mutation in *FKBP10* (OMIM: 607063, c.918-3C>G), which was located in the AOH region, and both parents were heterozygous for this mutation.

In a 2-year-old boy (Case 115) diagnosed with dilated cardiomyopathy, SNP array revealed a 12.513-Mb AOH located in 5q23.3q31.3. Parental verification confirmed no indication of UPD. Among multiple pathogenic genes in the AOH region, the *SLC22A5* gene (OMIM: 603377), which is the pathogenic gene of primary carnitine deficiency, inherited in an autosomal recessive pattern, was strongly suspected to be involved in the observed heart disease. As expected, gene mutation testing demonstrated that the boy carried a homozygous mutation in *SLC22A5* (c.760C>T), which was inherited from both parents, who were heterozygous for the mutation.

Outcome and follow-up

The outcomes of the 100 fetuses with AOH were as follows: 48 term births (of which three resulted in neonatal death), 24 TOPs, nine preterm births (of which two resulted in neonatal death), three selective reductions of twin pregnancies, three fetal deaths and one miscarriage, and 12 patients were lost to follow-up. A full-term neonate (Case 19) and a premature infant (Case 73) were found to have difficulty feeding and were diagnosed postnatally with intestinal atresia or dysplasia; they subsequently underwent surgery. One fetus (Case 36) had Blake's pouch cyst on prenatal ultrasound screening, and was delivered vaginally at term; the infant showed growth delay and hypertonia and was unable to walk at the age of 1.5 years. After excluding cases with incomplete follow-up information, the rate of adverse perinatal outcome (including TOP, neonatal death, fetal death, selective reduction and miscarriage) in fetuses with AOH that had ultrasound abnormalities was 62.5% (30/48), while it was 15.0% (6/40) in those that did not show positive ultrasound findings ($P < 0.001$).

Of the 35 non-fetal patients whose peripheral blood samples showed AOH, 19 had an abnormal phenotype (Table S3). The clinical phenotypes included growth delay ($n = 8$), intellectual disability ($n = 5$), autism ($n = 2$), congenital genitourinary malformation, ataxia, dilated cardiomyopathy, intraventricular myxoma, retinitis pigmentosa, primary infertility and sterility.

Based on our findings, we developed a proposed strategy for the management of fetuses with AOH detected by SNP array (Figure 2).

DISCUSSION

In this study, we summarize the chromosomal distribution and clinical features of fetuses diagnosed prenatally with AOH. The rate of AOH > 10 Mb in our population was 0.95%, whereas a rate of 0.5–28% has been reported in European general populations[15]. The distribution of AOH varies in each chromosome[4]. We found that AOH was more likely to occur in chromosomes X, 2 and 16; however, Wen *et al.*[16] found that AOH > 1 Mb was observed most frequently in chromosomes 8, 2, 6 and 10. This inconsistency could be attributed to the different sizes of the studied AOH. AOH occurs readily in regions of high linkage disequilibrium and low recombination[17]. Similarly, we observed that Xp11.23p11.1 and Xq13.1q21.1 were

Copyright © 2019 ISUOG. Published by John Wiley & Sons Ltd.

the most frequent regions for AOH, which were also described as recurrent AOH in the study of Wen *et al.*[16]. However, the clinical significance of AOH has rarely been reported.

AOH shows variable ultrasound presentations. Prenatal ultrasound screening failed to detect the common findings associated with specific AOH. Fifty-five percent of AOH carriers had ultrasound abnormalities, and multiple malformations were the most common finding. Indicative prenatal ultrasound findings can present in BWS and SRS[18,19]. Patients with UPD14 and UPD22 showed multiple ultrasound abnormalities, resulting in unfavorable outcomes[20–22]. In our study, on follow-up, fetuses with ultrasound abnormalities showed a 4.2-fold higher rate of adverse perinatal outcome than did those without abnormalities. This suggests that, as ultrasound phenotypes provide important information for predicting the fetal prognosis, fetuses with AOH should be carefully screened using structural ultrasonography. Furthermore, we found that 11% of fetuses with AOH were SGA and, among non-fetal cases, eight children with AOH had growth delay. The genetic factors of SGA are correlated with UPD of chromosomes 16, 14, 15, 7 and 2[10,23,24]. The possible mechanisms leading to SGA include imprinting disorders, recessive diseases or placental functional insufficiency caused by CPM[25]. We speculate that, as with UPD, AOH is one of the possible genetic factors contributing to SGA. Monitoring of fetal growth using ultrasound during pregnancy is important to prevent adverse outcome.

Imprinting effects should be considered particularly in UPD, and the association between UPD and the length of AOH should be evaluated. The significance of UPD depends on the involved region, the imprinted gene and its origin[7]. Clinically significant imprinting disorders have been reported previously in chromosomes 6, 7, 11, 14, 15 and 20[10,26]. It has been shown that UPD16 has variable outcome, from normal growth to delayed growth[27]. Remarkably, UPD can occur as heterodisomy or isodisomy, but SNP array can identify only isodisomy. In Case 57, SNP array detected a 9.002-Mb AOH on chromosome 16; however, it was later confirmed as maternal UPD16 combined with a 78.32-Mb heterodisomy. As the length of AOH also depends on probe distribution and the selection of different array platforms, the AOH region may appear to be smaller or larger than its actual length. We speculated that defining an absolute cut-off level of 10 Mb may lead to some important information being missed. When AOH (even if less than 10 Mb) occurs in chromosomes indicating suspected pathogenicity, UPD should be verified in parental samples.

AOH provides information for exploring recessive diseases. We successfully detected three AOH cases with homozygous mutations of a recessive pathogenetic gene, accounting for their abnormal phenotypes. AOH inherited from both parents indicates that the parents share a relatively close common ancestor, and offspring from consanguinity have more AOHs than those from outbred populations[28]. The risk of homozygous mutations increases with the length of the AOH[29]. Thus, WES is recommended for investigating homozygous mutations, especially in fetuses with malformations detected on ultrasound or those resulting from consanguinity.

Discordant results between NIPT, karyotyping and SNP array can be attributed to AOH. Studies have reported that CPM can cause positive NIPT results with normal fetal karyotype[30,31], and have shown that AOH in 15q and 22q can yield positive cell-free DNA analysis results[32,33]. As fetal DNA fragments in the maternal circulation originate from the placenta[34], NIPT results reflect more accurately placental chromosomal conditions rather than those of the fetal chromosomes. When abnormal karyotypes are confined to the placenta and fetal cells develop chromosomal rescues, AOH may occur. Further placental studies could help confirm the diagnosis.

Our data have implications for the genetic counseling of patients with AOH (Figure 2). For fetuses of non-consanguineous parents, we propose the following evaluation strategy: (1) ultrasound structural anomalies should be carefully screened for by qualified sonographers, and fetal growth should be monitored throughout pregnancy; (2) parental verification should be performed to confirm UPD and the origin of AOH; (3) WES can be used to explore the homozygous mutation in recessive pathogenic genes; (4) after birth, placental studies should be performed by multisite sampling to confirm the CPM; and (5) effective and long-term follow-up is warranted. For fetuses born to consanguineous parents, ultrasound screening and trio WES (for the fetus and parents) should be performed first. If WES fails to explain the phenotype, further verification of UPD and placental studies are recommended. Further multicenter studies, more reliable databases and associated studies on mechanisms could accumulate data for exploring the contribution of AOH to diverse phenotypes.

Our study has some limitations. First, it was a retrospective single-center study, and incomplete follow-up information may have led to the number of late-onset adverse phenotypes being underestimated. Second, the origin of AOH was not identified by parental samples in most cases and no placental studies were performed, thus failing to determine whether it was UPD or CPM. Moreover, the incidence of AOH in our cohort may be higher than the incidence in the general population, because we recruited women undergoing invasive prenatal diagnosis for all indications including ultrasound anomalies and advanced maternal age, who commonly have a higher risk for fetal chromosomal abnormalities than does the general population[35].

In conclusion, we describe here the occurrence and prenatal clinical features of AOH. Multiple methods may aid the comprehensive evaluation of AOH and the management of affected pregnancies, including serial ultrasound examinations, parental verification, WES, placental studies and postnatal follow-up.

Copyright © 2019 ISUOG. Published by John Wiley & Sons Ltd.

ACKNOWLEDGMENTS

We thank all families that participated in our study, which was supported by the National Natural Science Foundation of China (No. 81741015).

REFERENCES

1. Miller DT, Adam MP, Aradhya S, Biesecker LG, Brothman AR, Carter NP, Church DM, Crolla JA, Eichler EE, Epstein CJ, Faucett WA, Feuk L, Friedman JM, Hamosh A, Jackson L, Kaminsky EB, Kok K, Krantz ID, Kuhn RM, Lee C, Ostell JM, Rosenberg C, Scherer SW, Spinner NB, Stavropoulos DJ, Tepperberg JH, Thorland EC, Vermeesch JR, Waggoner DJ, Watson MS, Martin CL, Ledbetter DH. Consensus statement: chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. *Am J Hum Genet* 2010; **86**: 749–764.
2. Hillman SC, McMullan DJ, Hall G, Togneri FS, James N, Maher EJ, Meller CH, Williams D, Wapner RJ, Maher ER, Kilby MD. Use of prenatal chromosomal microarray: prospective cohort study and systematic review and meta-analysis. *Ultrasound Obstet Gynecol* 2013; **41**: 610–620.
3. Levy B, Sigurjonsson S, Pettersen B, Maisenbacher MK, Hall MP, Demko Z, Lathi RB, Tao R, Aggarwal V, Rabinowitz M. Genomic imbalance in products of conception: single-nucleotide polymorphism chromosomal microarray analysis. *Obstet Gynecol* 2014; **124**: 202–209.
4. Kirin M, McQuillan R, Franklin CS, Campbell H, McKeigue PM, Wilson JF. Genomic runs of homozygosity record population history and consanguinity. *PLoS One* 2010; **5**: e13996.
5. Campbell H, Carothers AD, Rudan I, Hayward C, Biloglav Z, Barac L, Pericic M, Janicijevic B, Smolej-Narancic N, Polasek O, Kolcic I, Weber JL, Hastie ND, Rudan P, Wright AF. Effects of genome-wide heterozygosity on a range of biomedically relevant human quantitative traits. *Hum Mol Genet* 2007; **16**: 233–241.
6. Ku CS, Naidoo N, Teo SM, Pawitan Y. Regions of homozygosity and their impact on complex diseases and traits. *Hum Genet* 2011; **129**: 1–15.
7. Robinson WP. Mechanisms leading to uniparental disomy and their clinical consequences. *Bioessays* 2000; **22**: 452–459.
8. Yamazawa K, Ogata T, Ferguson-Smith AC. Uniparental disomy and human disease: an overview. *Am J Med Genet C Semin Med Genet* 2010; **154C**: 329–334.
9. Lapunzina P, Monk D. The consequences of uniparental disomy and copy number neutral loss-of-heterozygosity during human development and cancer. *Biol Cell* 2011; **103**: 303–317.
10. Kotzot D. Prenatal testing for uniparental disomy: indications and clinical relevance. *Ultrasound Obstet Gynecol* 2008; **31**: 100–105.
11. Kearney HM, Thorland EC, Brown KK, Quintero-Rivera F, South ST, Working Group of the American College of Medical Genetics Laboratory Quality Assurance Committee. American College of Medical Genetics standards and guidelines for interpretation and reporting of postnatal constitutional copy number variants. *Genet Med* 2011; **13**: 680–685.
12. Slovak ML, Theisen A, Shaffer LG. Human Chromosome Nomenclature: An Overview and Definition of Terms. In *The Principles of Clinical Cytogenetics*, Gersen S, Keagle M (eds) Springer: New York, NY, USA, 2013; 23–49.
13. Li H, Durbin R. Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinformatics* 2009; **25**: 1754–1760.
14. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL, ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015; **17**: 405–424.
15. McQuillan R, Leutenegger AL, Abdel-Rahman R, Franklin CS, Pericic M, Barac-Lauc L, Smolej-Narancic N, Janicijevic B, Polasek O, Tenesa A, Macleod AK, Farrington SM, Rudan P, Hayward C, Vitart V, Rudan I, Wild SH, Dunlop MG, Wright AF, Campbell H, Wilson JF. Runs of homozygosity in European populations. *Am J Hum Genet* 2008; **83**: 359–372.
16. Wen J, Comerford K, Xu Z, Wu W, Amato K, Grommisch B, DiAdamo A, Xu F, Chai H, Li P. Analytical validation and chromosomal distribution of regions of homozygosity by oligonucleotide array comparative genomic hybridization from normal prenatal and postnatal case series. *Mol Cytogenet* 2019; **12**: 12.
17. Gibson J, Morton NE, Collins A. Extended tracts of homozygosity in outbred human populations. *Hum Mol Genet* 2006; **15**: 789–795.
18. Bruce S, Hannula-Jouppi K, Puoskari M, Fransson I, Simola KO, Lipsanen-Nyman M, Kere J. Submicroscopic genomic alterations in Silver–Russell syndrome and Silver–Russell-like patients. *J Med Genet* 2010; **47**: 816–822.
19. Eggermann T, Brioude F, Russo S, Lombardi MP, Bliek J, Maher ER, Larizza L, Prawitt D, Netchine I, Gonzales M, Gronskov K, Tumer Z, Monk D, Mannens M, Chrzanowska K, Walasek MK, Begemann M, Soellner L, Eggermann K, Tenorio J, Nevado J, Moore GE, Mackay DJ, Temple K, Gillessen-Kaesbach G, Ogata T, Weksberg R, Algar E, Lapunzina P. Prenatal molecular testing for Beckwith–Wiedemann and Silver–Russell syndromes: a challenge for molecular analysis and genetic counseling. *Eur J Hum Genet* 2016; **24**: 784–793.
20. Watanabe T, Go H, Kagami M, Yasuda S, Nomura Y, Fujimori K. Prenatal findings and epimutations for paternal uniparental disomy for chromosome 14 syndrome. *J Obstet Gynaecol Res* 2015; **41**: 1133–1136.
21. Chen CL, Lee CN, Lin MW, Hsu WW, Tai YY, Lin SY. Prenatal diagnosis of paternal uniparental disomy for chromosome 14 using a single-nucleotide-polymorphism-based microarray analysis: A case report. *J Formos Med Assoc* 2019; **118**: 739–742.
22. Li X, Liu Y, Yue S, Wang L, Zhang T, Guo C, Hu W, Kagan KO, Wu Q. Uniparental disomy and prenatal phenotype: Two case reports and review. *Medicine (Baltimore)* 2017; **96**: e8474.
23. Yingjun X, Zhiyang H, Linhua L, Fangming S, Linhuan H, Jinfeng T, Qianying P, Xiaofang S. Chromosomal uniparental disomy 16 and fetal intrauterine growth restriction. *Eur J Obstet Gynecol Reprod Biol* 2017; **211**: 1–7.
24. Carmichael H, Shen Y, Nguyen TT, Hirschhorn JN, Dauber A. Whole exome sequencing in a patient with uniparental disomy of chromosome 2 and a complex phenotype. *Clin Genet* 2013; **84**: 213–222.
25. Wilkins-Haug L, Quade B, Morton CC. Confined placental mosaicism as a risk factor among newborns with fetal growth restriction. *Prenat Diagn* 2006; **26**: 428–432.
26. Eggermann T, Soellner L, Buiting K, Kotzot D. Mosaicism and uniparental disomy in prenatal diagnosis. *Trends Mol Med* 2015; **21**: 77–87.
27. Kotzot D, Utermann G. Uniparental disomy (UPD) other than 15: phenotypes and bibliography updated. *Am J Med Genet A* 2005; **136**: 287–305.
28. Woods CG, Cox J, Springell K, Hampshire DJ, Mohamed MD, McKibbin M, Stern R, Raymond FL, Sandford R, Malik Sharif S, Karbani G, Ahmed M, Bond J, Clayton D, Inglehearn CF. Quantification of Homozygosity in Consanguineous Individuals with Autosomal Recessive Disease. *Am J Hum Genet* 2006; **78**: 889–896.
29. Bittles A. Consanguinity and its relevance to clinical genetics. *Clin Genet* 2001; **60**: 89–98.
30. Pan M, Li FT, Li Y, Jiang FM, Li DZ, Lau TK, Liao C. Discordant results between fetal karyotyping and non-invasive prenatal testing by maternal plasma sequencing in a case of uniparental disomy 21 due to trisomic rescue. *Prenat Diagn* 2013; **33**: 598–601.
31. Van Opstal D, van Veen S, Joosten M, Diderich KEM, Govaerts LCP, Polak J, van Koetsveld N, Boter M, Go ATJI, Papatsonis DNM, Prinsen K, Hoefsloot LH, Srebniak MI. Placental studies elucidate discrepancies between NIPT showing a structural chromosome aberration and a differently abnormal fetal karyotype. *Prenat Diagn* 2019; **39**: 1016–1025.
32. Schwartz S, Kohan M, Pasion R, Papenhausen PR, Platt LD. Clinical experience of laboratory follow-up with noninvasive prenatal testing using cell-free DNA and positive microdeletion results in 349 cases. *Prenat Diagn* 2018; **38**: 210–218.
33. Bunnell M, Zhang C, Lee C, Bianchi DW, Wilkins-Haug L. Confined placental mosaicism for 22q11.2 deletion as the etiology for discordant positive NIPT results. *Prenat Diagn* 2017; **37**: 416–419.
34. Taglauer ES, Wilkins-Haug L, Bianchi DW. Review: cell-free fetal DNA in the maternal circulation as an indication of placental health and disease. *Placenta* 2014; **35** (Suppl): S64–S68.
35. Nicolaides KH. Screening for chromosomal defects. *Ultrasound Obstet Gynecol* 2003; **21**: 313–321.

SUPPORTING INFORMATION ON THE INTERNET

The following supporting information may be found in the online version of this article:

Table S1 Ultrasound findings, results of genetic testing and outcome of 100 fetuses diagnosed prenatally with AOH

Table S2 Results of SNP array in 11 fetuses diagnosed with AOH following miscarriage

Table S3 Clinical features and results of SNP array in 35 non-fetal cases with AOH

Copyright © 2019 ISUOG. Published by John Wiley & Sons Ltd.