Clinical utility of chromosomal microarray analysis and whole exome sequencing in fetuses with oligohydramnios

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Research Article

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Abstract

Objective: To evaluate the clinical utility of chromosomal microarray analysis (CMA) and whole exome sequencing (WES) in fetuses with oligohydramnios.

Methods: In this retrospective study, 126 fetuses with oligohydramnios at our center from 2018 to 2021 were reviewed. Results of CMA and WES were analyzed.

Results: One hundred and twenty-four cases had CMA performed and 32 cases had WES performed. The detection rate of pathogenic/likely pathogenic (P/LP) copy number variants (CNVs) by CMA was 1.6% (2/124). WES revealed P/LP variants in 21.8% (7/32) of fetuses. Six (85.7%, 6/7) fetuses showed an autosomal recessive inheritance pattern. Three (42.9%, 3/7) variants were involved in the renin-

angiotensin-aldosterone system (RAAS), which are the known genetic causes of autosomal recessive renal tubular dysgenesis (ARRTD).

Conclusion: CMA has low diagnostic utility for oligohydramnios. WES is an efficient approach and should be recommended in cases of oligohydramnios.

Introduction

Oligohydramnios is a decreased in the volume of amniotic fluid, diagnosed in 0.5–5.5% of all pregnancies [1]. It is associated to a high rate of pregnancy complications and increased fetal morbidity and mortality [2]. Etiologies of oligohydramnios are diverse and vary according to the timing of diagnosis. Causes of oligohydramnios include fetal abnormalities (most often involving the genitourinary tract), placental insufficiency, ruptured membranes and maternal drug exposure [3].

Numerous genetic disorders can present with oligohydramnios. Chromosomal abnormalities associated with oligohydramnios include aneuploidy, such as trisomy 16, and partial chromosomal aberrations such as Wolf-Hirschhorn syndrome [4, 5]. In addition, oligohydramnios can also be accompanied with single-gene defects, such as autosomal recessive renal tubular dysgenesis(ARRTD), autosomal recessive polycystic kidney disease(ARPKD), Pierson syndrome, Fraser syndrome and many other conditions [6, 7, 8, 9]. Recently, Chromosomal microarray analysis (CMA) has been recommended to replace conventional karyotype analysis as the first-tier detection method when fetal abnormalities are detected by ultrasound [10, 11]. However, with further researches in recent years, it is found that the detection rate of CMA in oligohydramnios is low [12]. With increasing availability of sequencing technology, decreasing costs and improved speed of bioinformatic analytical pipelines, whole-exome sequencing (WES) has been recently introduced for both research purposes and clinical use. Recent studies show that WES has the ability to provide genetic diagnoses ranging from 9.1–32% for the fetuses with structural anomalies [13, 14, 15]. Nevertheless, the comprehensive WES assessment of fetuses with oligohydramnios is limited.

In the present study, we reviewed the clinical and molecular findings of 126 fetuses with oligohydramnios to explore the yield of CMA and WES. This study aimed to provide scientific guidance for prenatal diagnosis and consultation for oligohydramnios.

Materials And Methods

Ethics approval

This is a retrospective analysis of pregnancies diagnosed with oligohydramnios in the Guangdong women and children hospital from January 2018 to December 2021.The study was approved by the Ethical Committee of the Guangdong Women and Children hospital and samples from the patients were obtained according to the Helsinki Declaration. Written informed consent for genetic testing was obtained from all patients.

Subjects

Fetal ultrasound anatomy scans were routinely performed for pregnant women by senior sonographers using GE E8 ultrasound machines (General Electric Healthcare, US). Amniotic fluid was evaluated by calculating the amniotic fluid index (AFI) and the deepest vertical pool (DVP). Oligohydramnios was defined as AFI < 5cm or DVP < 2cm [16]. The exclusion criteria were as follows: oligohydramnios resulting from ruptured membranes or maternal drug exposure.

CMA

Fetal DNA was obtained from amniotic fluid or umbilical cord blood. DNA was extracted using the QIAamp DNA Blood Mini Kit (Qigen, Germany). Microarray analyses were performed using a high-resolution genotyping single nucleotide polymorphism microarray, Affymetrix CytoScan 750K Array (Affymetrix, Santa Clara, CA, USA). CNVs were identified based on associated records of the human reference genome 37(NCBI37hg19) of the National Center for Biotechnology Information.

WES

Subsequently genomic DNA samples form parent–fetus trio were subjected to WES using Illumina Nextera Rapid Capture Exome Kit for library preparation. Sequencing was performed with Illumina NovaSeq 6000 (Illumina, Inc., San Diego, CA, USA). Variants were classified according to the American College of Medical Genetics and Genomics (ACMG) guidelines by the laboratory. All the selected variants were then classified as pathogenic, likely pathogenic, VOUS, likely benign or benign. Only variants related to the phenotype were reported. The pathogenic variants detected by the WES analysis were then verified by Sanger sequencing.

Statistical Analysis

Statistical analysis was performed with the SPSS 24.0 (IBM). Quantitative variables are expressed as the mean ± standard deviation, and categorical variables are expressed as the frequency and percentage. Differences between categorical variables were analysed using chi-square test or Fisher's exact test. $P < 0.05$ was considered statistically significant.

Results

Characteristics of patients

During the study period we identified 126 cases of oligohydramnios. Of all cases in the study, 62 (49.2%, 62/126) cases were isolated oligohydramnios, while 64 (50.8%, 64/126) were non-isolated (fetuses had additional ultrasonography anomalies such as soft markers or structural abnormality). The mean age at diagnosis was 26.8 ± 4.1weeks (range 17–35 weeks), and the mean maternal age was 29.5 ± 4.8 years (range 20–43 years). Overall, 19.8% (25/126) of prenatal samples were performed following amniocentesis and 80.2% (101/126) following cordocentesis sampling.

Results of CMA

Of the 124 cases with CMAs performed, one pathogenic CNVs and one likely pathogenic CNVs were identified. Overall, the detection rate of P/LP CNVs was 1.6% (2/124). In addition, four (3.2%, 4/124) VOUS findings were noted (Table 1).

Fetus 1 was presenting with oligohydramnios at 17 weeks. CMA revealed a duplication on chromosome 10q23.31q26.3 and a deletion at 4p16.3p16.2 in the fetus, which indicated that the karyotype of one of the parents may be balanced chromosomal translocation. CMA revealed a 3.0-Mb duplication on chromosome 9q34.11q34.13 in fetus 2 with oligohydramnios and IUGR.

Results of WES

WES was successfully performed in 32 fetuses. Pathogenic/likely pathogenic CNVs were identified in 7 fetuses, yielding a detection rate of 21.8% (7/32). De novo variants were identified in 1 cases (14.3%, 1/7), and 6 (85.7%, 6/7) variants were functional homozygous or compound heterozygous mutations in accordance with their recessive patterns of inheritance. Three (42.9%, 3/7) variants were involved in the renin-angiotensin-aldosterone system (RAAS), which are the known genetic causes of ARRTD (Table 2).

Case 1 was a fetus presenting with severe oligohydramnios, hyperechogenic kidneys and enlarged kidneys. AGT exon 3-4 (E3_E4 del) homozygous deletion was detected by WES, indicating parental heterozygosity. In case 2, ultrasound showed oligohydramnios and hyperechogenic kidneys. WES showed a heterozygous point variation c.598C>T (p.Q200*) (novel variant) from the mother and the heterozygosity E3_E4 del from the father, forming a compound heterozygosity. In case 3, ultrasound showed severe oligohydramnios. WES revealed a homozygous point variation c.1028G>A in the ACE gene. All the three fetuses were finally diagnosed as ARRTD.

We also found a homozygous point variation c.199-10T>G in the SLC25A20 gene in case 4. Mutation of SLC25A20 is causative for Carnitine-acylcarnitine translocase deficiency (CACTD). Case 5 presented with oligohydramnios, hyperechogenic kidneys and enlarged kidneys. WES revealed a compound heterozygous mutation in PKHD1, a gene associated with PKD4. Case 6 had bilateral renal dysplasia and hypoplastic nasal bone in addition to oligohydramnios. WES revealed a compound heterozygous mutation in FRAS1. Mutations in the FRAS1 gene may cause Fraser syndrome. Case 7 carried a heterozygous mutation of (c.1406_1413dup8) in the HNF1B gene. Mutations in this gene cause renal cysts and diabetes syndrome. Ultrasound showed severe oligohydramnios, bilateral renal dysplasia and enlarged heart.

In addition, one fetus showed genetic variants that were not P/LP, but merited further clinical and molecular investigations, and it was classified as VOUS variant. The total proportion of fetuses with VOUS variants is 3.1% (1/32).

Breakpoint PCR detection:

WES found fetus 1 and fetus 2 had the same E3_E4 del in AGT gene. Then both point mutation and deletion were confirmed by Sanger sequencing.

Located in the upstream of intron 2 primer IN2F (5 '- CAGGTCTGCCACTGCC

TTCTTAC -3') and downstream primer in intron 5 IN5R (5'- AGACTCTGTGGGCT

CTCTCATCC - 3') amplified a shorter product than the wild fragment in the samples with E3_4del. The wild fragment was 4280bp, and 2870bp region deleted (chr1:230,839,696-230,842,565, GRCh37/hg19). The PCR product containing the breakpoint was 1419bp band, which included a 9bp insertion (Fig 2A,2B), the same as the previously reported deletion type [17].

Prenatal diagnosis of the fetus was performed by amniocentesis at the third pregnancy of Family 1. Breakpoint PCR detection was performed in amniotic fluid DNA, and no 1419bp fragment was found, replaced by the wild fragment length (Fig 2B). It suggested that the fetus did not inherit the AGT deletion from the parents. Follow-up amniotic fluid monitoring of the fetus was normal.

Discussion

Oligohydramnios is a potential indicator of underlying disease due to association with numerous pathologies including chromosomal abnormality and single-gene defects. However, the cause of oligohydramnios is uncertain in many cases. Studies have showed that CMA has the ability to detect clinically significant CNVs in 6–8% of pregnancies with fetal structural abnormalities[10]. However, Singer A, et al. reviewed 50 prengnancies with oligohydramnios and found that

the yield of CMA did not differ from the control population[12]. In our study, P/LP CNVs were identified in two fetuses, yielding a detection rate of 1.6%. These results were consistent with previous research and further support that CMA has low diagnostic utility for oligohydramnios.The most likely explanation for the low yield of CMA in oligohydramnios is that most genetic causes are due to single-gene defects, rather than CNVs.

Case 1 harbored a 44.9-Mb duplication in chromosome 10q23.31-q26.3 and a 5.5-Mb deletion in 4p16.3p16.2. The deletion in chromosome 4p16.3p16.2 that was linked to Wolf-Hirschhorn syndrome, a syndrome characterized by typical facial appearance, intellectual disability, developmental delay, seizures, skeletal anomalies, congenital heart defect and urinary tract malformations[18]. The duplication in chromosome 10q23.31-q26.3 included many OMIM genes associated with typical facial appearance, developmental delay and intellectual disability. This result suggests that one parent may be a balanced translocation carrier. Case 2 had a 3.0-Mb duplication in chromosome 9q34.11q34.13 that contained 21 OMIM genes, which are associated with mild/moderate intellectual disability, dysmorphic features, and hypotonia in infancy. Nevertheless, the demonstration of these two pathogenic CNVs in our studies might be incidental, as both of them have no clear association with oligohydramnios.

In this study, P/LP variants were identified in 21.8% (7/32) of the fetuses, which indicates a high diagnostic yield of WES for prenatal molecular diagnoses of oligohydramnios. Six (85.7%, 6/7) fetuses showed an autosomal recessive inheritance pattern. WES offers obvious advantages in improving the detection rate than CMA, which improves pregnancy management, prenatal counseling and recurrence risk assessment for future pregnancies. Our study suggests WES is an efficient approach for prenatal diagnosis of pregnancies with oligohydramnios and should be recommended in case of oligohydramnios in clinical practice.

Three (42.9%, 3/7) variants were involved in the renin-angiotensin-aldosterone system (RAAS), which are the known genetic causes of ARRTD. ARRTD is a rare inherited disease of abnormal development of renal tubular, resulting in early onset and perinatal death, due to persistent hypotension, severe renal failure, and bronchopulmonary dysplasia [19]. It was first reported in two stillborn siblings who had developed fetal anuria in 1983 [20]. Oligohydramnios/anhydramnios is the most common sign that is noted in fetuses with ARRTD. It is caused by mutations in genes (ACE, AGT, AGTR1 and REN) encoding components of the RAAS [21, 22]. Our findings highlights that pathogenic variants in genes (ACE, AGT, AGTR1 and REN) are the most common generic lesion for oligohydramnios.

Recently, Ma GC et al. reported a fetus with ARRTD, which showed anhydramnios and invisible urinary bladder since the second trimester, genetic analysis identified a novel, biparental-origin homozygousc 857 − 619_1269 + 243 Delins TTGCCTTGC mutation in the AGT gene [17]. In this study, we also found two fetuses (fetus 1 and 2) had the same E3_E4 del, missing 2870bp and 9bp insertion in AGT, which resulted in a trunked protein (1-292 amino acids) caused by the AGT serpin domain encoding exons skipped. In silico modeling proves that the interaction between mutant AGT and rein is diminished. Our results were the same as the previously reported deletion type. Tseng MH et al. reviewed a series of 6 Taiwanese individuals with ARRTD from 6 unrelated families diagnosed by renal histology. All the 6 patients were also homozygous for AGT E3_E4 del. The heterozygous allele frequency of this variant was about 1.2% (6/500), suggesting that ARRTD may not be very rare in Taiwan [23]. In addition, deletion CNV of different sizes in the same region will be found in the Database of Genomic Variants (esv2657518, essv6399317), suggesting that CNV in this region may also be mediated by other mechanisms, such as Nonallelic Homologous Recombination(NAHR), replicative mechanisms, long interspersed element (LINE)-mediated retrotransposition or mobile element insertions (MEIs) and nonhomologous end-joining (NHEJ). Among these, NAHR and replicative mechanisms play a crucial role in explaining various germline and somatic rearrangement events. There are some repeat masker sequences near the microdeletion region detected, which may mediate the occurrence of the initial small CNV through the above mechanism. We need more experiments or cases to prove this.

In addition, we found a homozygous point variation c.199-10T > G in the SLC25A20 gene in a fetus with severe oligohydramnios. Mutations in this gene cause CACTD. CACTD is a rare autosomal recessive metabolic disorder of long-chain fatty acid oxidation. The clinical manifestations are mainly neuropsychiatric abnormalities, cardiomyopathy, arrhythmias, skeletal muscle damage and liver dysfunction, renal tubular fatty changes, etc. Some patients have symptoms such as asphyxia, dyspnea, and oliguria[24]. Our study is the first study that evaluates the prenatal phenotype of fetus diagnosed with CACTD. The result identify a further expansion of the prenatal presentation of CACTD and contribute to the genetic diagnosis and counseling of this disorder.

Conclusions

Our data demonstrated CMA has low diagnostic utility for oligohydramnios, while WES offers obvious advantages in improving the detection rate. Although future prospective studies with larger sample sizes are warranted, given the increased diagnostic yield, our study suggests WES should be recommended in case of oligohydramnios in clinical practice.

Limitations of this study mainly stem from its retrospective nature of data acquisition. In addition, the sample size was small, which may have limited some of our comparisons. We hope that further prospective investigation by multicenter and prospective research may be warranted to evaluate the utility of WES for prenatal diagnosis for pregnancies with oligohydramnios.

Declarations

Acknowledgments

We are grateful to the patient and her family who participated in this study. We obtained consent for the publication of the patient's photograph and results.

Author Contributions

Xiaomei Shi and Hongke Ding: collected data and wrote the manuscript. Chen Li and Juan Zhu: data collection and manuscript editing. Ling Liu and LiHua Yu and: researched data. Jing Wu: project development and and manuscript writing. All

authors read and approved the final manuscript.

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Availability of data and materials

All data generated during and/or analyzed during the current study are available upon request by contact the corresponding author.

Ethics approval and consent to participate

The study was approved by the Ethical Committee of the Guangdong Women and Children hospital. All procedures were applied following the Declaration of Helsinki, as well as international and national laws, guidelines and regulations. Signed informed consent was obtained from all the pregnant women enrolled in this study.

Consent for publication

All authors consented to publish.

Competing interests

The authors declare no conficts of interest.

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Tables

Table 1. P/LP CNVs and VOUS in fetuses with oligohydramnios by CMA.

NO	Other ultrasound findings	CMA results	Size of CNVs	CNV information	Classification	outcomes
1	/	4p16.3p16.2(68,346-5,601,945)x1 10q23.31q26.3(90,517,129-135,426,386)x3	5.5Mb 44.9Mb	Wolf-Hirschhorn syndrome and 10q23.31-q26.3 duplication	P	TOP
2	FGR	9q34.11q34.13(132,123,319-135,111,146)x3	3.0Mb	9q34 duplication	LP	ceaserian section at 30^{+5}w, male, 0.96KG. Normal newborn examination
3	/	22q13.2(41,306,166-42,869,987)x3	1.56Mb	22q13.2 duplication	VOUS	ceaserian section at 37^{+2}w, female,2.1KG. Normal newborn examination
4	bilateral renal agenesis	4q21.1q21.21(77,204,232-80,520,004)x1	3.3Mb	4q21 deletion syndrome	VOUS	TOP
5	/	1q21.1(145406788_145809118)x3	402kb	1q21.1 duplication (BP2-BP3)	VOUS	ceaserian section at 37^{+3}w, male, 2.8KG. Normal newborn examination
6	/	13q12.12(23519917_24947363)x3	1.4Mb	13q12.12 duplication	VOUS	ceaserian section at 37^{+5}w, female, 2.75KG. Normal newborn examination

Abbreviations:

P/LP:pathogenic/likely pathogenic; CNVs: copy number variants; VOUS: variants of unknown significance

FGR: fetal growth restriction; TOP: termination of pregnancy

Table 2. P/LP variants and VOUS in fetuses with oligohydramnios by WES.

NO	Other ultrasound findings	Gene	Transcript	Nucleotide change	Amino acid change	ACMG Classification	Origin	Disease (OMIM ID)	Inheritance model	Pregnancy outcome
1	hyperechogenic kidneys,enlarged kidneys	AGT	NM_000029	Exon 3-4 deletion	/	LP	Parents	ARRTD (267430)	AR	TOP
2	hyperechogenic kidneys	AGT	NM_000029	c.598C>T and Exon 3-4 deletion	p.Q200*	LP	Parents	ARRTD (267430)	AR	TOP
3	/	ACE	NM_000789	c.1028G>A	p.W343*	P	Parents	ARRTD (267430)	AR	TOP
4	/	SLC25A20	NM_000387	c.199-10T>G	/	P	Parents	CACTD (212138)	AR	TOP
5	hyperechogenic kidneys,enlarged kidneys	PKHD1	NM_1386944	c.9901G>T and c.11314C>T	p.Glu3301* and p.Arg3772*	LP	Parents	PKD4 (263200)	AR	TOP
6	bilateral renal dysplasia and hypoplastic nasal bone	FRAS1	NM_025074	c.10748G>A and c.3152-2A>G	p.W3583*	LP	Parents	Fraser syndrome 1 (219000)	AR	TOP
7	bilateral renal dysplasia and enlarged heart	HNF1B	NM_000458	c.1406_1413dup8	p.V472Cfs*36	P	De novo	renal cysts and diabetes syndrome (137920)	AD	TOP
8	FGR, enlarged heart	GDI1	NM_001493	c.310G>A	p.V104M	VOUS	De novo	XLID41 (300849)	XLD	TOP

P: pathogenic; LP: likely pathogenic; VOUS: variants of unknown significance

ARRTD: autosomal recessive renal tubular dysgenesis; CACTD: Carnitine-acylcarnitine translocase deficiency;

PKD4: Polycystic kidney disease 4; FGR: fetal growth restriction; XLID41: Intellectual developmental disorder, X-linked 41;

AD: Autosomal dominant; AR: Autosomal recessive; XLD: X-linked dominant; TOP: termination of pregnancy

Figures

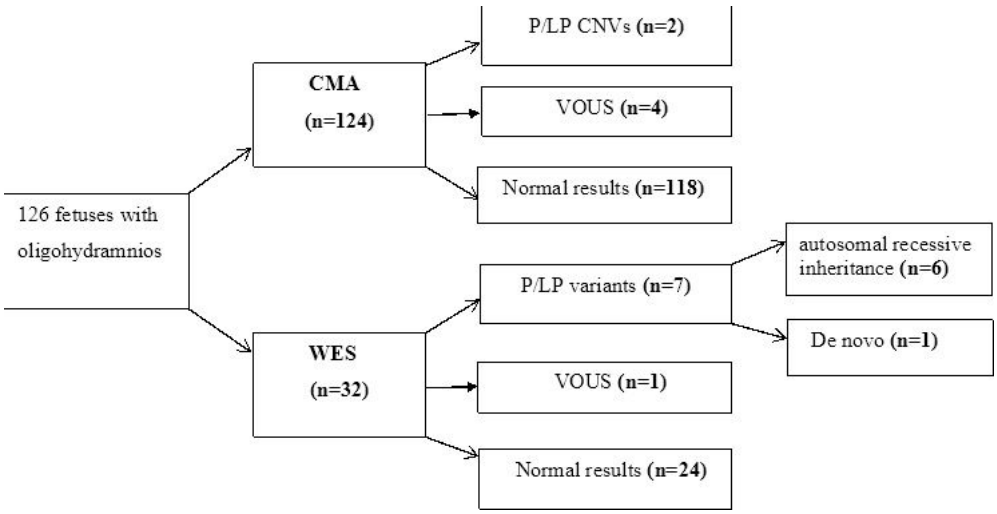

Figure 1

Prenatal diagnosis for fetuses with oligohydramnios by CMA and WES

CMA: Chromosomal microarray; WES: whole exome sequencing

P/LP: pathogenic/likely pathogenic; CNVs: copy number variants; VOUS: Variants of unknown or uncertain significance

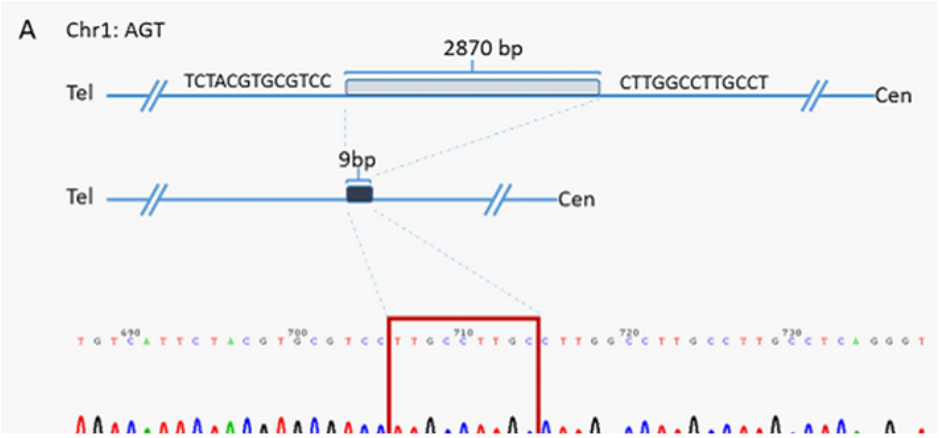

Figure 2

The diagram of the AGT gene deletion fragments and the PCR products sequencing result containing breakpoint

A: The wild fragment diagram of the first line (light gray bar is E3_E4 del region), the second is 2870bp deletion replaced by a 9bp insertion, the third lane shows the sequence containing the breakpoint, and the last is the point mutation of fetus 2 (red arrow).

B: With 3 lanes as a unit, from left to right are: PCR products containing the breakpoint (bands with 1419bp length), AGT exon 2 amplification products (wild product bands), exon 3 amplification products (carriers have products, patient has nothing). Lane 1-12 are pedigree results of fetus 1, lane 1-3 are father's, 4-6 are mother's, 7-9 are abnormal fetus's, 10-12 are prenatal diagnosis of the third fetus (lane 10 is the wild fragment). Lane 13-24 are the results of fetus 2, 13-15 are father's, 16-18 are maternal results (no breakpoint product was observed, this may be nonspecific amplification band), 19-21 are abnormal fetus's.