Analysis of Correlation between Whole Exome Sequencing and Ultrasound Examination in Prenatal diagnosis of Fetal Skeletal Dysplasia

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Abstract

Fetal skeletal dysplasia is a disease that is difficult to distinguish these types of diseases during the fetal period. Due to the difficulty of fetal ultrasound diagnosis, the severity of fetal skeletal dysplasia is extremely difficult to assess. For this condition, we analyzed 79 fetal samples of skeletal dysplasia from the third affiliated hospital of Zhengzhou University, China from August 2018 to April 2020, which had undergone prenatal whole exome sequencing(WES). By comparing the results of whole-exome sequencing and fetal ultrasound test results, we find that the fetal short limb phenotype found in the range of FL<-4.0SD or HL<-4.0SD through ultrasound test is closely related to FGFR3 gene mutation , and the correlation is stronger when accompanied by macrocephaly. We also find that the fetal limb curved phenotype is closely related to COL1A1 gene mutation. At the same time, we find that nasal dysplasia during fetal period is also a common phenotype of abnormal results detected by whole exome sequencing. Overall, our research shows that WES has different detection rates for various skeletal abnormalities according to the different types of ultrasound detection results, which provides a meaningful guidance for clinical diagnosis of fetal skeletal dysplasia.

Keywords

Whole exome sequencing; fetal skeletal dysplasia; ultrasound examination; *FGFR3* ; *COL1A1*

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Introduction

Fetal skeletal dysplasia is a disease of osteochondrocytes with strong clinical variability(Grannum and Hobbins, 1983; Liu, et al., 2019). Due to their rarity, it is difficult to distinguish these types of diseases during the fetal period(Yang, et al., 2019). The diagnosis of fetal skeletal dysplasia can be done through prenatal ultrasound evaluation(Yang, et al., 2019). Skeletal dysplasia often causes a reduction in bust size and leads to lung dysplasia, so fetal skeletal dysplasia is often fatal. However, due to the difficulty of fetal ultrasound diagnosis, the severity of fetal skeletal dysplasia is extremely difficult to assess(Tang, et al., 2020). The accuracy of conventional ultrasound on fetal skeletal dysplasia is no more than 40%(Tang, et al., 2020). Misdiagnosis can lead to erroneous information about the risk of relapse and delay optimal fetal management.

Using fetal imaging as a guide, a personalized prenatal genetic examination strategy can be selected. Current options include chromosome karyotype analysis, chromosome fluorescence in situ hybridization experiments,

chromosome microarray analysis, whole exome sequencing etc(Petrovski, et al., 2019). According to the 2010 revision of the Nosology and Classification of Genetic Skeletal Disorders, 456 diseases were divided into 40 groups according to molecular, biochemical and radiological standards(Warman, et al., 2011). Among them, 316 bone-related diseases are related to the mutation of one or more genes in 226 different genes, which provides a basis for the molecular genetic diagnosis of fetal skeletal development abnormalities(Warman, et al., 2011). Two recent studies have shown that in the assessment of large scale prospectively ascertained cohort of fetal with skeletal anomalies, the diagnostic rates of skeletal dysplasia are 10/65 (15.4%)(Lord, et al., 2019) and 8/34 (24%)(Petrovski, et al., 2019), respectively.

In our study, we analyzed 79 fetal samples of skeletal dysplasia from the third affiliated hospital of Zhengzhou University, China from August 2018 to April 2020, which had undergone prenatal whole exome sequencing(WES). Our research aims to explore the genetic types of fetal skeletal dysplasia in China, and explore the correlation between results by WES and phenotype of skeletal dysplasia in the fetal period by ultrasound, with a view to providing a theoretical basis for early implementation of birth defect intervention and reproductive risk assessment.

Materials and Methods

Pregnant women Enrollment,specimens and DNA preparation

We selected a total of 79 pregnant women from the third affiliated hospital of Zhengzhou University, China, from August 2018 to April 2020, who had undergone whole exome sequencing due to fetal ultrasound suggesting skeletal dysplasia. Sample entry requirements: pregnant women between 18-36 weeks of gestation, ultrasound examination shows femur length <-2SD or humerus length <-2SD or other skeletal development abnormalities: such as nasal bone dysplasia, limb bone deformity, skull or clavicle development Exception etc. We performed amniocentesis on the enrolled pregnant women for subsequent DNA extraction. Afterwards, we used QIAGEN 69504 blood and tissue DNA extraction kit for DNA extraction from amniotic fluid, peripheral blood of pregnant women and their husbands.

Whole exome sequencing(WES) and CNV analysis

The genomic DNA of amniotic fluid was broken into random fragments and purified, and whole genome exome capture was performed for sequencing library preparation. The Illumina Hiseq XTen sequencer was used to perform double-ended high-throughput sequencing with a length of 150 bp. The raw data obtained by the sequencing were quality-controlled, and the basic data were analyzed and filtered to remove the linker sequence and repeat sequence. Data were analyzed using the Anno variant site detection system, the XYGeneRanger variant site annotation interpretation system.

Variant annotation and interpretation

The pathogenicity assessment and data interpretation rules of mutation are based on the guidelines of the American College of Medical Genetics and Genomics (ACMG) (Richards, et al., 2015). By querying 1000genomes, ExAC and gnomAD, we excluded gene mutations whose frequency of the population are more than 1% and removed non-functional mutation sites (such as synonymous mutations, non-coding region mutations, etc.).We performed gene function prediction (using software such as SIFT, Polyphen2, CADD, etc.) and clinical symptoms comparison. At last, we searched related disease databases and related references, and finally found candidate gene mutation sites for family verification. The variant annotation databases which were used include Human Genome hg19/GRCh37, RefSeq, dbSNP, 1000 Genomes phase3, ExAC, and gnomAD and the interpretation databases which were used include DGV, DECIPHER, OMIM, UCSC, ClinVar, HGMD and PubMed.

Family verification by sanger sequencing

Genetic modification of the mutations was performed on the fetus, pregnant women and their husbands. According to the exome of the mutation site, the primers needed for sequencing the synthetic DNA fragments were designed, and the DNA of the fetus, pregnant women and their husbands were PCR-amplified. Method

of sequencing was performed by Sanger sequencing and the sequencing results were compared with the results of whole exome sequencing.

Results

Correlation between positive results by WES and clinical phenotypes by ultrasound

Among 79 pregnant women from the third affiliated hospital of Zhengzhou University, China, from August 2018 to April 2020, 25 cases that were positive by WES were detected,whose detection rate was 31.6%.Among these cases, the detection rate of cases with only FL<-4.0SD or only HL<-4.0SD was 41.6% (5/12). The detection rate of cases with both FL<-4.0SD and HL<-4.0SD is 60% (3/5). In cases of -4.0SD<FL<-2.0SD or -4.0SD<HL<-2.0SD, the detection rate by WES was 29.4% (5/17).However,in these cases where exsists -4.0SD<FL<-2.0SD or -4.0SD<HL<-2.0SD, with long bone bending, the detection rate by WES was 100% (2/2). In cases of FL<-2.0SD or HL<-2.0SD, the detection rate by WES is 20% (1/5), where the only one positive case besides FL<-2.0SD and HL<- 2.0SD, there exsists also femoral curvature. In cases with only long bone bending, the detection rate by WES is 50% (2/4). In cases of nasal bone dysplasia, the detection rate by WES is 80% (4/5). It is worth mentioning that cases of only microcephaly have not been detected positive by WES. Only 1 of 9 cases of fetal hand or foot deformity were positive by WES (11.1%).(Table .1)

Correlation between abnormal results of *FGFR3* gene sequenced by WES and clinical phenotypes by ultrasound

Among 25 cases that are positive by WES, 7 cases are caused by*FGFR3* gene mutation, accounting for 28%.Among them, 6 cases are*FGFR3* gene c.1138G>A mutation, and 1 case is*FGFR3* gene c.1620C>A mutation. In these 7 cases, the ultrasound test results of almost all cases show FL<-4.0SD or HL<-4.0SD(Only one case of short limbs with ambiguous data),among which 3 cases of ultrasound test results also show macrocephaly, accounting for 42.8%.Besides, all genetic variations are de novo.(Table.2)

Correlation between abnormal results of *COL1A1* gene sequenced by WES and clinical phenotypes by ultrasound

Among 25 cases that are positive by WES, 3 cases are caused by*COL1A1* gene mutation, accounting for 12%. In these 3 cases, the ultrasound test results of 2 cases show FL>-4.0SD or HL>-4.0SD,),among which all of 3 cases of ultrasound test results show fetal limbs slightly curved, accounting for 100%. Besides, One of the three cases has a *COL1A1* gene variant inherited from the pregnant husband, and the rest of COL1A1 gene variant are de novo. (Table.3)

Correlation between abnormal results of other gene sequenced by WES and clinical phenotypes by ultrasound

Among 25 cases that are positive by WES,11 cases are caused by other gene mutation accounting for 44%. In these 11 cases, there are 2 cases caused by *RUNX2* gene mutation, both of which have nasal bones not shown by ultrasound. In addition, there are 2 cases due to *COL2A1*gene mutation, both of which have short limbs by ultrasound**.**The other 7 cases are caused by *ARSE* gene mutation, *EFTUD2*gene mutation, *SCN4A* gene mutation, *COL1A2* gene mutation,*PEX7* gene mutation, *NEB* gene mutation and *FGFR2*gene mutation. Ultrasound test results related to these 7 genetic variations are mainly manifested by phenotypes other than short limbs, such as microcephaly, bilateral foot varus, the femur is slightly curved, abnormal ossification of both humerus and femur, fingers merge , toes merge, etc.(Table.4)

Correlation between abnormal results ofcopy number variation (CNV) sequenced by WES and clinical phenotypes by ultrasound

Among 25 cases that are positive by WES,4cases are caused by copy number variation accounting for 16%. Among these 4 cases, 3 cases were caused by chromosome copy number variation, and 1 case was chromosomal aneuploidy (Trisomy 21). 3 cases of chromosomal copy number variation were all deletions, and their phenotypes by ultrasound varied. 1 case of trisomy 21 has a phenotype for nasal bone dysplasia by ultrasound.(Table.5)

Table.1 Correlation between positive results by WES and clinical phenotypes by ultrasound

ID	Age(years)	Gestational age(weeks)	Phenotype by ultrasound
1	31	22^{+2}	Fetal vertebral fusion
2	28	25	HL<-3.2SD,FL<-2.1SD; both femurs are slightly curved
3	28	23	Fetal punctate achondroplasia
4	35	26	Fetal Multi-finger
5	25	24	Curved fetal osteogenesis
6	26	21	Poor skull development, nasal bones not shown, poor ossification of the lumbar
7	32	33	HL<-5.9SD,FL<-5.9SD;metaphyseal enlargement
8	30	21	Right femur slightly curved
9	30	24	The long bones of the limbs are obviously short, the alveolar bone is flat, and t
10	28	27^{+4}	HL<-4.0SD,FL<-4.0SD;femur slightly curved
11	31	26^{+1}	Fetal nasal and jaw abnormalities
12	18	25^{+3}	BPD>+2.0SD;fetal bilateral ventricular widening
13	27	30^{+6}	FL<-3.4SD,HL<-2.8SD
14	26	31	BPD>+2.0SD,FL<-4.0SD,HL<-4.0SD
15	30	29	The fetus is greater than 3 weeks of the gestational week, and the femurs are sl
16	28	25^{+2}	FL<-2.0SD,HL<-2.0SD; both femurs are slightly curved
17	34	26^{+5}	FL<-3.9SD,HL<-4.3SD; uneven ossification of some vertebral bodies
18	27	27^{+4}	FL<-3.0SD,HL<-3.0SD
19	28	27^{+5}	BPD>+2.0SD,FL<-4.0SD,HL<-4.0SD
20	26	27	FL<-2.0SD,HL<-2.0SD
21	29	18^{+5}	Fetal limbs slightly curved
22	16	20	FL<-4.9SD,HL<-4.7SD
23	32	32	Fetal limbs are short
24	25	30^{+2}	FL<-3.0SD, Single umbilical artery
25	23	27	HL<-2.6SD,HC<-2.8SD
26	37	25^{+5}	AC<-2.0SD,HL<-2.0SD,FL<-2.0SD
27	20	31	FL<-3.12SD,HL<-3.12SD
28	29	24^{+6}	Short limb development
29	25	30^{+6}	HL<-2.9SD,FL<-2.9SD
30	32	25	FL<-6.0SD
31	28	28	FL<-2.7SD,HL<-3.9SD
32	33	32	BPD>+3.7SD, HC>+2.8SD,AC>+3.3SD
33	25	27^{+2}	FL<-3.0SD,HL<-3.0SD
34	30	26^{+4}	BPD>+5.0SD,
35	25	23^{+4}	Fetal short femur
36	27	26	Split vertebra of fetal half vertebra, bilateral valgus, abnormal posture of both
37	30	33^{+4}	FL<-2.0SD
38	26	35^{+3}	BPD<-5.0SD
39	23	27^{+4}	BPD<-3.0SD,HC<-2.0SD
40	30	33^{+3}	FL<-3.0SD,HL<-2.0SD
41	30	26^{+2}	Bilateral foot varus
42	31	31	BPD<-2.75SD
43	28	30	HC>+2.5SD
44	26	27	HC<-2.0SD,AC<-2.0SD,HL<-2.0SD,FL<-3.0SD
45	38	26	FL<-2.0SD,HL<-2.0SD, nose bones not shown
46	29	23^{+2}	Multi-finger
47	31	24	Right foot inversion
48	34	33	HL<-2.9SD,FL<-3.5SD

ID	Age(years)	Gestational age(weeks)	Phenotype by ultrasound
49	27	23^{+6}	Bilateral foot varus,cervical thoracic spine bends backward
50	23	19^{+1}	FL<-3.0SD, The femur is slightly curved and the long bone is dysplastic
51	27	22^{+5}	FL<-4.0SD,HL<-3.7SD
52	35	30^{+5}	BPD<-3.0SD,HC<-2.0SD
53	30	25^{+5}	Multiple vertebral ossification
54	33	26^{+3}	Skull halo is not regular, bilateral temporal bones are slightly depressed,HC<-2
55	26	25	Dysplasia of fourth and fifth toes of right foot
56	28	25^{+5}	FL<-3.0SD,HL<-2.9SD
57	26	33	AC<-2.4SD,FL<-3.9SD,HL<-4.1SD
58	27	27	FL<-4.0SD
59	29	32	BPD<-4.0SD,HC<-3.0SD,AC<-2.0SD,FL<-2.0SD
60	31	23^{+1}	Nose root is flat, nose frontal angle is enlarged, nose and lip angles are acute, n
61	32	26^{+3}	Spinal cone low
62	31	18^{+2}	BPD<-3.3SD,FL<-5.0SD
63	27	30^{+4}	FL<-3.0SD,HL<-3.0SD
64	42	27	BPD<-2.5SD,FL<-4.0SD
65	15	24^{+5}	Multi-finger
66	28	23	Multi-finger
67	23	23	HC<-2.0SD,HL<-8.0SD,FL<-5.0SD;Abnormal ossification of both humerus and
68	30	24^{+3}	Bilateral foot varus
69	36	23^{+5}	BPD<-2.0SD,AC<-3.0SD,FL<-5.0SD
70	29	23	FL<-8.0SD,HC<-4.9SD
71	29	23	L1 half cone, T11 split vertebra
72	29	30^{+3}	FL<-4.0SD,HL<-2.0SD
73	30	26	Nasal bone dysplasia
74	29	31^{+1}	Fusion vertebra
75	31	28	BPD<-3.2SD,HC<-2.5SD
76	29	24^{+3}	Bilateral foot varus,Abnormal hand posture
77	21	27	Fingers merge, toes merge, temporal depression. Pregnant women also have fin
78	41	31	FL<-3.69SD
79	26	30	HC>+2.4SD,AC<-0.34SD,FL<-4.2SD

Table.2 Correlation between abnormal results of *FGFR3* gene sequenced by WES and clinical phenotypes by ultrasound

ID	Phenotype by ultrasound	Reference sequence	Sequencing results by clinical WES	Genetic subregion	Heterogeneity	Chromosomal Loci	Mutation type	Variant type(ACMG evidence levels)	Sanger verification
7	HL<-5.9SD,FL<-5.9SD;metaphyseal enlargement	NM_-000142.4	*FGFR3*:c.11[illegible]G>A(p.G3[illegible]R)	[illegible]	[illegible]	chr4:1806119-1806119	Pathogenic	PS2_-VeryS-trong+PS1+PS3+PS4+Strong+PM2+PP4	Paternal Wild
14	BPD>+2.0SD,HL<-4.0SD,HL<-4.0SD	NM_-000142.4	*FGFR3*:c.11[illegible]G>A(p.G3[illegible]R)	[illegible]	[illegible]	chr4:1806119-1806119	Pathogenic	PS2_-VeryS-trong+PS1+PS3+PS4+Strong+PM2+PP4	Wild

ID	Phenotype by ultrasound	Reference sequence	Sequencing results by clinical WES	Genetic subregion	Heterogeneity	Chromosomal Loci	Mutation type	Variant type(ACMG evidence levels)	Sanger verification
19	BPD>+2.0SD,FL<-4.0SD,HL<-4.0SD	NM_000142.4	*FGFR3*:c.1620C>A(p.N540K)	EX10	Het	chr4:1807371-1807371	Pathogenic	PS1+PS2+PM1+PM2+PP3	Wild
23	Fetal limbs are short	NM_000142.4	*FGFR3*:c.1138G>A(p.G380R)	EX9	Het	chr4:1806119-1806119	Pathogenic	PS2_VeryStrong+PS1+PS3+PS4+Strong+PM2+PP4	Wild
30	FL<-6.0SD	NM_000142.4	*FGFR3*:c.1138G>A(p.G380R)	EX9	Het	chr4:1806119-1806119	Pathogenic	PS2_VeryStrong+PS1+PS3+PS4+Strong+PM2+PP4	Wild
72	FL<-4.0SD,HL<-2.0SD	NM_000142.4	*FGFR3*:c.1138G>A(p.G380R)	EX9	Het	chr4:1806119-1806119	Pathogenic	PS2_VeryStrong+PS1+PS3+PS4+Strong+PM2+PP4	Wild
79	HC>+2.4SD,AC<-0.34SD,FL<-4.2SD	NM_000142.4	*FGFR3*:c.1138G>A(p.G380R)	EX9	Het	chr4:1806119-1806119	Pathogenic	PS2_VeryStrong+PS1+PS3+PS4+Strong+PM2+PP4	Wild

Table.3 Correlation between abnormal results of *COL1A1* gene sequenced by WES and clinical phenotypes by ultrasound

ID	Phenotype by ultrasound	Reference sequence	Sequencing results by clinical WES	Genetic subregion	Heterogeneity	Chromosomal Loci	Mutation type	Variant type(ACMG evidence levels)	Sanger verification
2	HL<-3.2SD,FL<-2.1SD; both femurs are slightly curved	NM_000088.3	*COL1A1*:c.856G>A(p.G286D)	EX16	Het	chr17:48273852-48273852	Pathogenic	PS1+PS2+PM1+PM2+PP3	Paternal
16	FL<-2.0SD,HL<-2.0SD; both femurs are slightly curved	NM_000088.3	*COL1A1*:c.3235G>A(p.G1079S)	EX44	Het	chr17:48265483-48265483	Likely pathogenic	PS1+PM2+PP3+PP4	Het

ID	Phenotype by ultrasound	Reference sequence	Sequencing results by clinical WES	Genetic subregion	Heterogeneity	Chromosomal Loci	Mutation type	Variant type(ACMG evidence levels)	Sanger verification
21	Fetal limbs slightly curved	NM_-000088.3	*COL1A1*:c.1[illegible]G>A(p.G[illegible]D)	Exon	Het	chr17:48272460-48272460	Pathogenic	PS2+PM2+PM5+PP3+PP4	Positive

Table.4 Correlation between abnormal results of other gene sequenced by WES and clinical phenotypes by ultrasound

ID	Phenotype by ultrasound	Reference sequence	Sequencing results by clinical WES	Genetic subregion	Heterogeneity	Chromosomal Loci	Mutation type	Variant type(ACMG evidence levels)	Sanger verification
6	Poor skull development, nasal bones not shown, poor ossification of the lumbar vertebral body of the spine, poor ossification of the sacral caudal vertebral arch and vertebral body	NM_-001024630.3	*RUNX2*:c.93[illegible]946del(p.T311fs)	Exon7	Het	chr6:45480052-45480069	Pathogenic	PVS1++PS2+PM2	Paternal Positive

ID	Phenotype by ultrasound	Reference sequence	Sequencing results by clinical WES	Genetic subregion	Heterogeneity	Chromosomal Loci	Mutation type	Variant type(ACMG evidence levels)	Sanger verification
11	Fetal nasal and jaw abnormalities	NM_-000047.2	*ARSE*:c.331C>T(p.R111C)	Exon5	Hemi	chrX:2871283-2871283	Likely pathogenic	PM1+PM2+PM5+PP3	Wild
17	FL<-3.9SD,HL<-4.3SD; uneven ossification of some vertebral bodies	NM_-001844.4	*COL2A1*:c.1120-2A>G	Intron22	Het	chr12:48380228-48380228	Pathogenic	PVS1+PS2+PM2+PP3	Wild
25	HL<-2.6SD,HC<-2.8SD	NM_-004247.3	*EFTUD2*:c.1705C>T(p.R569X)	Exon17	Het	chr17:42937814-42937814	Pathogenic	PVS1+PS2+PM1+PM2+PP3	Wild
28	Short limb development	NM_-001844.4	*COL2A1*:c.1246G>A(p.G416R)	Exon29	Het	chr12:48377887-48377887	Likely pathogenic	PS2+PM2+PP3+PP4	Wild
45	FL<-2.0SD,HL<-2.0SD, nose bones not shown	NM_-001024630.3	*RUNX2*:c.569G>T(p.R190W)	Exon3	Het	chr6:45399744-45399744	Pathogenic	PS1+PS2+PM1+PM2+PP3	Wild
49	Bilateral foot varus,cervical thoracic spine bends backward	NM_-000334.4	*SCN4A*:c.4361G>A(p.R1454Q)	Exon24	Het	chr17:62019281-62019281	Likely pathogenic	PM1+PM2+PM5+PP3	Wild
		NM_-000334.4	*SCN4A*:c.481G>A(p.E161K)	Exon3	Het	chr17:62049497-62049497	Likely pathogenic	PM1+PM2+PM5+PP3	Wild

ID	Phenotype by ultrasound	Reference sequence	Sequencing results by clinical WES	Genetic subregion	Heterogeneity	Chromosomal Loci	Mutation type	Variant type(ACMG evidence levels)	Sanger verification
50	FL<-3.0SD, The femur is slightly curved and the long bone is dysplastic	NM_-000089.3	*COL1A2*:c.2261G>T(p.G754V)	Exon37	Het	chr7:94049926-94049926	Pathogenic	PS1+PS2+PM2+PP3	Wild
67	HC<-2.0SD,HL<-8.0SD,FL<-5.0SD;Abnormal ossification of both humerus and femur, poor ossification of cervical vertebra	NM_-000288.3	*PEX7*:c.283T>G(p.W95G)	Exon3	Het	chr6:137147551-137147551	Likely Pathogenic	PM1+PM2+PM3+PP3	Het
		NM_-000288.3	*PEX7* :c.408dup(p.V137Cfs*16)	Exon4	Het	chr6:137166820-137166820	Likely Pathogenic	PVS1+PM2	Wild
76	Bilateral foot varus,Abnormal hand posture,	NM_-001271208.1	*NEB*:c.1569+5G>A	Exon17	Het	chr2:152553146-152553146	VUS	PM2+PM3+PP3	Het
		NM_-001271208.1	*NEB*:c.2278C>T(p.Q760*)	Exon24	Het	chr2:152547273-152547273	Likely Pathogenic	PVS1+PM2	Wild

ID	Phenotype by ultrasound	Reference sequence	Sequencing results by clinical WES	Genetic subregion	Heterogeneity	Chromosomal Loci	Mutation type	Variant type(ACMG evidence levels)	Sanger verification
77	Fingers merge, toes merge, temporal depression,.Pregnant women also have finger merge and toe merge	NM_-000141.4	*FGFR2*:c.755C>G	Exon7	(p.S252W)	chr10:123279677	Pathogenic	PM6_-VeryStrong+PS3+PM2+	Wild

Table.5 Correlation between abnormal results of copy number variation (CNV) sequenced by WES and clinical phenotypes by ultrasound

ID	Phenotype by ultrasound	Chromosome	Micro-deletion/duplication results and significance	Size(bp)	Related genes	Related diseases
5	Curved fetal osteogenesis	ND	1q21.1-q21.2 deletion, chr1:146631138-147415663	0.78M	*GJA5;GJA8*	1q21.1recurrent microdeletion(susceptibility locus for neurodevelopmental disorders
24	FL<-3.0SD, Single umbilical artery	ND	2q35-q36.1 deletion, chr2:219247086-222436973	3.19M	*EPHA4*	2q35-q36.1 deletion
40	FL<-3.0SD,HL<-2.0SD	ND	Xp22.33-p21.2 deletion, chrX:2700101-30327485	27.63M	*ARSE;NLGN4X;STS;ANOS1;GPR CLCN4;MID1;HCCS;AMELX;FRM TRAPPC2;OFD1;FANCB;PIGA;A NHS;CDKL5;RS1;PHKA2;ADGRG PDHA1;RPS6KA3;CNKSR2;SMP MBTPS2;SMS;PHEX;PTCHD1;KI EIF2S3;PDK3;POLA1;ARX;IL1RA*	

ID	Phenotype by ultrasound	Chromosome	Micro-deletion/duplication results and significance	Size(bp)	Related genes	Related diseases
		ND	Xq21.31- q28 deletion, chrX:88008410-154774942	66.77M	*DIAPH2;PCDH19;SRPX2;TIMM8 BTK;GLA;HNRNPH2;GPRASP2; PLP1;SERPINA7;PIH1D3;PRPS1 MID2;COL4A6;COL4A5;ACSL4;A ; CHRDL1;PAK3;DCX;ALG13;PLS UBE2A;UPF3B;RNF113A;NDUFA CUL4B;C1GALT1C1;GLUD2*	NS
73	Nasal bone dysplasia	Trisomy 21	21q11.2-q22.3 Duplication, chr21:14982544-48084291	33.1M	ND	Down's Syndrome

Discussion

By studying the WES test results of 79 pregnant women from the third affiliated hospital of Zhengzhou University from August 2018 to April 2020, the overall detection rate of skeletal abnormalities was 31.6%. This is higher than the previously reported detection rate (15.4%(Lord, et al., 2019) and 24%(Petrovski, et al., 2019)). This may be due to our stricter requirements for the ultrasound test results of the enrolled cases. Interestingly, we find that for different types of skeletal abnormalities, the detection rate through WES varies greatly. Among them, WES has a higher detection rate for short limbs. Especially when FL<-4.0SD or HL<-4.0SD, the detection rate can rise to 41.6%. However, when the fetal has short limbs with other bone abnormal phenotypes, the detection rate will be higher. For example, when the limbs are short with bone curved, the WES detection rate can reach 100%; when the limbs are short with nasal bone dysplasia, WES detection rate can reach 80%. On the contrary, if the phenotype of short limbs is not detected by ultrasound testing and only other skeletal abnormal phenotypes exists, such as only fetal hand or foot deformities, the WES detection rate will be very low, only 11.1% . It may be that achondroplasia is the cause for the most of skeletal abnormalities (Pauli, 2019), which is also the most common form of inherited disproportionate short stature(Waller, et al., 2008). In our study, through ultrasound,46 of 79 fetal skeletal abnormalities had clinical manifestations of short limbs, accounting for 58.2%, consistent with previous reports(Georgoulis, et al., 2011). This shows that the diagnosis of achondroplasia is the key to the diagnosis of fetal skeletal abnormalities.

Through our research, we find that for the diagnosis of fetal*FGFR3* -related achondroplasia, the WES detection rate is highly correlated with the results of ultrasound testing of the fetal limb shortness and severity. Through our research, we find that the diagnosis of fetal *FGFR3* -related achondroplasia(Wynn, et al., 2007) accounts for the highest proportion of all skeletal abnormalities, reaching 28%. Almost all the ultrasound test results of fetal achondroplasia have been detected with FL<-4.0SD or HL<-4.0SD, suggesting that FL<-4.0SD or HL<-4.0SD is the most critical basis for the diagnosis of achondroplasia by fetal ultrasound testing. At the same time, the detection of macrocephaly through ultrasound is also an important evidence for the diagnosis of fetal achondroplasia. Among the 7 cases of*FGFR3* -related achondroplasia, 6 cases were caused by *FGFR3*gene c.1138G>A mutation. *FGFR3* gene c.1138G>A mutation is the most common mutation in*FGFR3* -related achondroplasia, which accounts for more than 99% of all *FGFR3* -related achondroplasia together with *FGFR3*gene c.1138G>C mutation(Foldynova-Trantirkova, et al., 2012; Xue, et al., 2014). At this point, our research is consistent with these reports. In addition, we have also detected a case of *FGFR3*gene c.1620C>A mutation through WES, which is related to hypochondroplasia(Bellus, et al., 1995). *FGFR3* gene c.1620C>A mutation has been reported extensively(Deutz-Terlouw, et al., 1998)

and is considered to account for 50%-76% of FGFR3-related hypochondroplasia(Tarja, et al., 2012). Therefore, *FGFR3* gene c.1138G>A mutation and c.1620C>A mutation may be the two most common causes of skeletal abnormalities in the fetal period and require special clinical attention.

Osteogenesis imperfecta caused by the *COL1A1* gene is also a major cause of fetal skeletal dysplasia. The main feature of Osteogenesis imperfecta is multiple fractures usually caused by minor trauma(W, et al., 1985; Willing, et al., 1990). This may be manifested by the limbs being curved by ultrasound testing during the prenatal period. In our study, osteogenesis imperfecta due to *COL1A1* gene mutation was detected in the presence of limbs slightly curved through ultrasound in the fetal period, which suggests that the presence of this phenotype during the fetal period may be highly correlated with COL1A1-related osteogenesis imperfecta. In addition, *COL1A1* gene c.896G>A mutation and c.1301G>A mutation found in our research have not been reported in other studies before , which broadens the clinical understanding of this gene mutation. *COL1A1*gene c.3235G>A mutation, according to previous reports, shows that it has a highly variable phenotype in the family, and family members with this gene mutation can only show signs of disease without fractures(D., et al., 2012; Kaneko, et al., 2011). This may explain why in our study, the husband of the pregnant woman also has the heterozygous mutation of the gene but the phenotype was not abnormal.

In our study, 2 cases of cleidocranial dysplasia related to *RUNX2*gene mutation were detected, which were c.931_946del mutation and c.568C>T mutation. The main clinical features of cleidocranial dysplasia include persistently open skull sutures with bulging calvaria, hypoplasia or aplasia of the clavicles permitting abnormal facility in apposing the shoulders, wide pubic symphysis, short middle phalanx of the fifth fingers, dental anomalies, and often vertebral malformation(Pan, et al., 2017). It has been pointed out in previous reports that cleidocranial dysplasia can also be related to the phenotype of nasal bone loss(Pan, et al., 2017), which is consistent with our case study. In our study, some other genetic variations or chromosomal aneuploidies related to the nasal bone loss phenotype are also found, such as the *ARSE* gene c.331C>T variation and trisomy 21. *ARSE* gene mutation is related to X-linked recessive chondrodysplasia punctata, which is manifested as nasal dysplasia and distal phalanx dysplasia(Brunetti-Pierri, et al., 2003), which is consistent with the phenotype observed during fetal ultrasound testing. Trisomy 21 is the most frequent form of mental retardation caused by a microscopically demonstrable chromosomal aberration, is characterized by well-defined and distinctive phenotypic features and natural history(Zhu, et al., 2013). It has been reported that nasal bone dysplasia is a common detected phenotype of fetal trisomy 21(De Jong Pleij, et al., 2012), which is consistent with the phenotype of a trisomy 21 case found in our study. Therefore, in the diagnosis of fetal skeletal abnormalities, nasal bone dysplasia may be another typical indication of ultrasound abnormality in addition to short limbs.

In our study, some gene mutations related to skeletal abnormalities related to hand and foot abnormalities are also detected. For example,*SCN4A* gene c.4361G>A mutation(Gay, et al., 2010),*NEB* gene c.1569+5G>A and c.2278C>T compound heterozygous mutation(Lehtokari, et al., 2014), *FGFR2*gene c.755C>G mutation(Miraoui, et al., 2010). Most of the diseases related to these gene mutations mainly affect the function of the muscular system as the main cause(Coen A. C. Ottenheijm, 2009; Matthews, et al., 2011), and their detection rate in fetal skeletal abnormalities is low (only *FGFR2* gene c.755C>G mutation), but their detection can play an important guiding role in clinical diagnosis and treatment.

Conclusions

In conclusions, our research shows that the application of whole exome sequencing technology can significantly improve the systemic prenatal diagnosis of skeletal abnormalities, and according to the different types of ultrasound detection results, WES has different detection rates for various skeletal abnormalities. Through our research, it is shown that fetal short limbs are the best detection targets for WES to detect skeletal abnormalities. In addition, the fetal limbs curved and nasal bone dysplasia are also important clinical phenotypes that suggest genetic variation-related skeletal abnormalities. However, the genetic basis of bone diseases is still unknown in other respects, indicating that new genes or non-genetic factors may cause these diseases.

Data Availability statement

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

Conflict of interest

The authors declared that they have no conflicts of interest to this work.

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