Early Sonographic Findings for Suspecting *de novo* Single-gene Mutation

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ABSTRACT

Introduction: Currently, many obstetrics departments widely use prenatal genetic screening tests in the first trimester, including noninvasive prenatal genetic tests (NIPT), sonographic examination, and combined tests. However, many cases with *de novo* single-gene mutations are detected after birth. The introduction of next-generation sequencing (NGS) needs careful pretest and posttest counseling, informed consent, and trio-based sequencing of the fetus and parents. Next-generation sequencing turnaround time has been reduced, but it takes a long period from an ultrasound anomaly scan at 18–20 weeks to post-NGS counseling. Ideally, the starting point of the anomaly scan should be early. Still, it has not yet been concluded whether there are clear criteria for the fetal indication to NGS in the first trimester of pregnancy.

Aim: To investigate first-trimester sonographic findings in cases with nonfamilial *de novo* single-gene mutation and to discuss early fetal findings as criteria for prenatal NGS test.

Materials and methods: For five months between August and December in 2019, after sonographic examinations between 11 and 13 gestational weeks for all fetuses, chorionic villous sampling (CVS) was done for suspected cases. DNA was extracted immediately after CVS for a rapid test of quantitative fluorescence-polymerase chain reaction (QF-PCR) and further examinations, such as, microarrays and exome sequencing. Confirming normal G-banding results, followed by further examinations of exome sequencing.

Results: Seven cases with *de novo* single-gene mutations were detected by target exome sequencing; one case of Costello syndrome, one of Campomelic dysplasia, one of Cornelia de Lange syndrome, and four of Noonan syndrome. All of those gene mutations were confirmed by following Sanger sequencing. All seven cases had common sonographic findings, increased NT ≥ 5 mm, low-set ears, and micrognathia. Three Noonan syndrome cases with PTPN11 mutation had tachycardia, but Noonan with RAF1 mutation had normal heart rate. Limb abnormality or contracture was detected in one case of Noonan syndrome, Campomelic dysplasia, and Cornelia de Lange syndrome.

Conclusion: Nonfamilial *de novo* single-gene disorders can be suspected in the first trimester by ultrasound findings of NT ≥ 5 mm, low-set ears, and micrognathia from our case series. Further case studies will be required.

Keywords: Exome sequencing, Fetus, First-trimester, Single gene mutation, Ultrasound.

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INTRODUCTION

Currently, many obstetrics departments widely use prenatal genetic screening tests in the first trimester, including noninvasive prenatal genetic tests (NIPT), sonographic examination, and combined tests. *In vitro* fertilization (IVF) patients could select the preimplantation genetic test for aneuploidy (PGT-A), structural rearrangement (PGT-SR), and monogenic disorder (PGT-M) before embryo transfer. PGT-M^{1,2} is designed for cases with the familial genetic mutation, but it is no use for nonfamilial *de novo* single gene mutation of fetuses. Therefore, cases with *de novo* single-gene mutations are mostly detected after birth. In recent years, many research articles have been published on target or whole exome sequencing for suspected anomalous fetuses detected by prenatal ultrasound. However, the implementation of next generation sequencing (NGS) needs careful pretest and posttest counseling, informed consent, and trio-based sequencing of the fetus and parents. Prenatal NGS should be undertaken for the cases which have already had comprehensive genetic testing, such as, microarray analysis. Generally, fetal anomaly scan is done around 18–20 weeks of gestation. Next generation sequencing turnaround time has recently been reduced, but it takes a long period from an ultrasound anomaly scan to amniocentesis, comprehensive genetic testing, comprehensive test result disclosure, pretest genetic counseling, NGS, and posttest counseling. Ideally, the starting point of the anomaly scan should be early. Still, it has not yet been concluded

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whether there are clear criteria for the fetal indication to NGS in the first trimester of pregnancy.

AIM

To investigate first-trimester sonographic findings in cases with nonfamilial *de novo* single-gene mutation and to discuss early fetal findings as criteria for prenatal NGS test.

PATIENTS AND METHODS

For 5 months between August and December in 2019, after advanced sonographic examination between 11 and 13 gestational weeks, a total of 580 CVSs was performed. Excluding multiple

pregnancy (14 dichorionic twins, 3 monochorionic twin, and 1 dichorionic triplets), 547 CVSs for singleton were included in this study. The chorionic villous sample was divided into two for DNA extraction and cell culture. DNA was extracted immediately for a rapid test of quantitative fluorescence-polymerase chain reaction (QF-PCR) and further examinations, such as, microarrays and exome sequencing. The cell culture for approximately 14 days was taken for cytogenetic G-banding analysis. For cases strongly suspected of genetic disease, confirming normal G-banding results, followed by further examinations, is shown in Flowchart 1. The target exome sequencing (TES) used in this study was TruSight One Sequencing Panel (Illumina, Inc. San Diego, CA, USA), which provides 4,813 disease-associated genes. In all cases, a trio analysis of fetus, mother, and father was performed. In the cases with pathogenic TES results, first-trimester ultrasound findings were retrospectively investigated for future criteria for prenatal NGS indication.

Pretest education and counseling were given, and the written informed consent was obtained from all patients agreeing to participate in the study, which was approved by the CRIFM Institutional Review Boards (Reference No. CRI-IRB-011).

RESULTS

Flowchart 2 shows the examination pedigree of cases with 547 CVSs. In 505 cases with normal G-band result, 44 (8.71%) underwent CMA and eight cases (1.58%) directly for TES because of strong suspicion of genetic diseases. In 39 cases with normal CMA results, eight cases (20.5%) underwent TES. In a total of 16 cases undergoing TES, pathogenic *de novo* single-gene disorder was found in seven cases (43.8%); one case of Costello syndrome due to HRAS gene mutation, one of Campomelic dysplasia due to SOX9 gene mutation, one of Cornelia de Lange syndrome due to NIPBL gene mutation, and four of Noonan syndrome due to PTPN11 gene mutation (three cases) and RAF1 mutation (one case). All of those gene mutations were confirmed by following Sanger sequencing.

Figure 1 shows first-trimester ultrasound findings in two cases of Noonan syndrome. In all of four Noonan cases, increased NT,

micrognathia, and low-set ear were detected in the first trimester. Three cases of PTPN11-related Noonan had tachycardia, but RAF1-related Noonan had normal fetal heart rate. Figure 2 shows first-trimester ultrasound findings at 12 weeks (left figures) and follow-up scan images at 17 weeks in the case of Campomelic dysplasia (Case 3) due to SOX9 mutation. From 12 weeks, contracted and immovable bilateral lower extremities, as well as increased NT, micrognathia, and low-set ear were demonstrated. A follow-up scan at 17 weeks revealed the bowing femur, clubfoot, and oligodactyly as shown in the right images of Figure 2. Sonographic images of Costello syndrome (Case 1) due to HRAS mutation are shown in Figure 3. Increased NT, micrognathia, and low-set ear were observed but normal position of arms and hands. A follow-up scan at 20 weeks revealed contracted hands with overlapping fingers, which did not exist at 13 weeks of gestation. Figure 4 shows the early findings of Cornelia de Lange Syndrome (Case 6) due to the NIPBL mutation. The case had oligodactyly along with increased NT, severe micrognathia, and low-set ear. A follow-up scan at 15 weeks shows more distinguished micrognathia and oligodactyly.

Table 1 shows details of seven cases with *de novo* single-gene mutations; pathogenic gene, disease, gestational age for the first-trimester ultrasound screening, sonographic findings, G-band/CMA results, and pregnancy outcome. The turnaround time for TES was 7–10 days, and we could receive all reports between 15 and 20 weeks of gestation (average 17 weeks and three days). Therefore, patients could have posttest genetic counseling before the end of 20 weeks of gestation.

The mean maternal age was 36.86, but the mean egg age was 34.0. Three out of seven cases were in their 20s. Six cases had spontaneous pregnancy, and only Case 7 was pregnant by IVF of donor's egg. Case 7 was 49-year-old, and her husband was 58-year-old. After ten times of IVF failure with her eggs, she obtained a 29-year-old donor's egg, and embryo transfer was done after PGT-A.

Six couples determined termination of pregnancy after genetic counseling with disclosure of pathogenic TES results. One couple

Flowchart 1: Workflow from chorionic villus sampling to molecular genetic examination

Flowchart 2: Genetic analysis progression in 547 chorionic villous samplings for singleton. CVS, chorionic villus sampling; CMA, chromosomal microarray; TES, target exome sequencing **Fig. 1:** Two cases of Noonan syndrome due to PTPN11 gene mutation (Case 5) and RAF1 mutation (Case 7). Both cases undertook ultrasound screening at 12 weeks of gestation. Both cases had increased NT, micrognathia, and low-set ear

(Case 2) continued pregnancy, but intrauterine fetal death was confirmed at 26 weeks and five days of gestation.

All seven cases had common sonographic findings, increased NT ≥ 5 mm, low-set ears, and micrognathia. Three Noonan syndrome cases with PTPN11 mutation had tachycardia, but Noonan with RAF1 mutation had normal fetal heart rate. Limb abnormality or contracture was detected in one case of Noonan syndrome, Campomelic dysplasia, and Cornelia de Lange syndrome. Costello case had no limb abnormality in the first trimester, but contracted hands were conspicuous in the midgestation.

DISCUSSION

Many research papers had been published on prenatal exome sequencing for anomalous fetuses from 2014.^{3–17} Generally, whole exome sequencing (WES) was used but some authors utilized the specific gene panels for cardiac diseases⁹ or skeletal diseases.¹⁰ For fetal precision medicine, a genetic investigation by use of NGS methodology may be exceedingly helpful for decision-making, early intervention of treatment for some of genetic diseases.¹⁸ However, it is still controversial to introduce next generation sequencing into the prenatal diagnosis, because there are incidental findings, the variants of uncertain significance (VOUS), likely benign,

Fig. 2: Camptomelic dysplasia (Case 3) due to SOX9 mutation. Left figures at 12 weeks and 6 days. Severe micrognathia and low-set ear and contracture of lower extremities are demonstrated. Right figures at 17 weeks and 5 days. Bowing femur (right lower), clubfoot, and oligodactyly are detected at this stage

Fig. 3: Costello syndrome (Case 1) due to HRAS mutation. Left figures at 13 weeks and 1 day. Mild micrognathia and low-set ear are demonstrated. Finger contracture was not seen at this stage. Right figure at 20 weeks and 3 days. Contracted hands with overlapping fingers are seen

Fig. 4: Cornelia de Lange syndrome (Case 6) due to NIPBL mutation. Left figures at 12 weeks and 2 days. Severe micrognathia and low-set ear are demonstrated. Oligodactyly is also seen. Right figures at 15 weeks and 2 days. Same findings as at 12 weeks but are demonstrated more clearly

and likely pathogenic, that make genetic counseling difficult. The International Society for Prenatal Diagnosis (ISPD), the Society for Maternal Fetal Medicine (SMFM), and the Perinatal Quality Foundation (PQF) issued the joint statement on the use of NGS for fetal diagnosis in 2018, which includes points for consideration before and after NGS test. In the statement, they described the routine use of NGS for fetal diagnosis.¹⁹

According to most of the published data, the timing of the anomalous scan was not described or throughout gestation. Recently, the data on increased NT and genetic tests, including G-banding, CMA, and exome sequencing, were reported,²⁰ but there was no pathogenic case by WES analysis. The amount of prenatal DNA sample is limited and it may be unreasonable to adapt ultrasound findings of increased NT as an indication for WES/TES.

CONCLUSION

In our series of pathogenic TES results, we found the common ultrasound findings of NT ≥ 5 mm, low-set ears, and micrognathia in all cases and those three points may be considered as a fetal indication for further WES/TES analysis. Tachycardia and Turner-like findings may be one of the features for early detection of Noonan syndrome due to PTPN11 gene mutation. Limb abnormality or contracture may help for suspicion of genetic disease. As described above, early detection of fetal abnormalities and early completion of comprehensive genetic tests, such as, G-banding or CMA, gives parents time for their decision-making. We need a further case study to determine early fetal findings as criteria for the prenatal NGS test.

Table 1: Pathogenic gene and disease, early sonographic findings, and outcome in seven cases with *de novo* single-gene disorder

Case no	Maternal age	Pathogenic gene	Disease	GA for FTS											Followup scan	G-band result	CMA result	Outcome	
				NT (mm)		GE	PE	MG	LSE	TR	MR	Small NB	DV reverse	Tachy-cardia					Limbs
1	37	HRAS	Costello syndrome	13 (1)	9.1	+	-	+	+	+	+	+	+	-	-	Contracted hand overlapping finger at 20 weeks	46,XY	Negative	TOP
2	27	PTPN11	Noonan syndrome	13 (4)	17.5	-	-	+	+	+	+	+	+	Clenched hand, wrist contracture	AVSD	-	46,XY	Negative	IUFD (26w5d)
3	27	SOX9	Cam-pomelic dysplasia	12 (6)	6.3	-	-	+	+	+	+	-	-	Contracture of bilateral lower limbs	-	Bowing femur, clubfoot, oligodactyly at 17 weeks	46,XX	-	TOP
4	41	PTPN11	Noonan syndrome	11 (6)	8.2	+	-	+	+	-	-	Absent DV	+	-	-	-	46,XX	Negative	TOP
5	39	PTPN11	Noonan syndrome	12 (0)	13.9	+	+	+	+	+	-	+	+	-	VSD	-	46,XY	Negative	TOP
6	38	NIPBL	Cornelia de Lange syndrome	12 (2)	10.5	+	+	+	+	+	-	+	+	Hypo-plastic forearm, oligodactyly	VSD	-	46,XX	-	TOP
7	49 (29)*	RAF1	Noonan syndrome	12 (3)	5.0	+	-	+	+	+	-	-	-	-	-	-	46,XX	-	TOP

GA, gestational age; FTS, first-trimester screening; NT, nuchal translucency; GE, general edema; PE, pleural effusion; MG, micrognathia; LSE, low-set ear; TR, tricuspid regurgitation; MR, mitral regurgitation; NB, nasal bone; DV, ductus venosus; AVSD, atrioventricular septum defect; VSD, ventricular septum defect; TOP, termination of pregnancy; IUFD, intrauterine fetal death; CMA, chromosomal microarray; HRAS, v-Ha-ras Harvey rat sarcoma viral oncogene homolog; PTPN11, protein tyrosine phosphatase, nonreceptor type 11; SOX9, SRY-box transcription factor 9; NIPBL, Nipped B-like; RAF1, Raf-1 proto-oncogene, serine/threonine kinase

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