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Learning Curve of First-Trimester Detailed Cardiovascular Ultrasound Screening by Moderately Experienced Obstetricians in 3509 Consecutive Unselected Pregnancies with Fetal Follow-Up

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Abstract: Our primary objective was to assess the effectiveness of detailed cardiovascular ultrasound screening during the first trimester, which was performed by obstetricians with intermediate experience. We collected first-trimester fetal cardiac screening data from an unselected pregnant population at RMC-Fetal Medicine Center during a study period spanning from 1 January 2010, to 31 January 2015, in order to analyze our learning curve. A pediatric cardiologist performed a follow-up assessment in cases where the examining obstetrician determined that the fetal cardiac screening results were abnormal or high-risk. Overall, 42 (0.88%) congenital heart abnormalities were discovered prenatally out of 4769 fetuses from 4602 pregnant women who had at least one first-trimester cardiac ultrasonography screening. In total, 89.2% of the major congenital heart abnormalities (27 of 28) in the following fetuses were discovered (or at least highly suspected) at the first-trimester screening and subsequent fetal echocardiography by the pediatric cardiology specialist. Of these, 96.4% were diagnosed prenatally. According to our results, the effectiveness of first-trimester fetal cardiovascular ultrasound screening conducted by moderately experienced obstetricians in an unselected ('routine') pregnant population may reach as high as 90% in terms of major congenital heart defects, provided that equipment, quality assurance, and motivation are appropriate.

Keywords: cardiovascular ultrasound screening; learning curve; moderately experienced obstetricians; fetal follow up

1. Introduction

Moderate and severe congenital heart defects (CHD) requiring intensive postnatal cardiac care are the most frequent fetal malformations with an incidence of 6–8 per 1000 live births. If we consider all small-sized ventricular septal defects (VSD), bicuspid aortic valves, and other minor cardiac malformations, which potentially increase cardiac morbidity at later ages, this incidence may be as high as 7.5 per cent [1]. Fetal cardiac anomalies are responsible for 20% of intrauterine fetal demise and 20 to 30% of neonatal mortality.

Prenatal diagnosis of CHD may help the work of genetic counselors, contribute to a better understanding of the prognosis of affected fetuses, and reduce the psychological and physical risk of affected pregnant women [2–4]. In utero detection of CHD may improve neonatal morbidity and mortality as a result of prior organization of intensive postnatal cardiac care [5–9]. Fetal echocardiography in high-risk patients is usually performed by pediatric cardiologists. The high-risk pregnant population includes pregnant women with a positive family history of CHD, in vitro fertilization, maternal metabolic disorder, mainly diabetes and obesity, higher maternal age, autoimmune diseases (SLE, Sjögren), fetal teratogen exposure, and abnormal NT, TR, and DV on the first-trimester genetic ultrasound examination [10–15]. In this group of high-risk pregnant patients, the vast majority of severe CHD cases are diagnosed prenatally [16,17]. On the other hand, according to the literature, 90% of CHD is not present in the high-risk group and routine obstetric ultrasound screening of the low-risk pregnant population is generally ineffective in finding the majority of major fetal cardiac anomalies due to the time-consuming nature of the examination and the lack of the acquired expertise, which would be greatly improved after a proper learning process [18–23]. Aneuploidy screening in the 11 to 13 + 6-week window by Fetal Medicine Foundation (FMF)-certified obstetric sonographers resulted in improved detection rates of CHD in the first trimester [24].

Given the fact that it is the obstetric sonographer who initially evaluates the 11–13-week fetus, there is great demand for obstetricians to conduct early and comprehensive fetal heart scans [25–27].

Thanks to the widespread use of high-frequency ultrasound scanners and the introduction of novel Doppler techniques, obstetricians skilled in fetal cardiac scanning are able to visualize four-chamber views and ventricular outflow tracts of first-trimester fetuses in approximately 95 to 97% of cases [28–31]. A significant number of international investigational research groups have proved high effectiveness (>90% detection rate, DR) of prospective first-trimester extended fetal cardiac screening conducted by highly qualified obstetric sonographers in high-risk pregnant patients [28,32]. However, the current literature lacks sufficient information regarding the effectiveness of early fetal cardiac examinations performed by obstetricians with basic and intermediate experience in unselected (low- and high-risk), also referred to as 'routine' pregnant populations, and of the learning curve [31,33,34].

Our study's primary goal was to assess the efficacy of first-trimester prolonged cardiovascular ultrasonography screening carried out by obstetricians with a moderate level of experience (an examiner experienced in the differential diagnosis of regular first-trimester heart planes but less experienced in the differential diagnosis of irregular first-trimester heart planes (i.e., has seen few abnormal cases)) in consecutive cases of an unselected pregnant population. Our investigation's second goal was to examine and better understand the learning curve we saw throughout the course of the five-year study period with reference to the sonographic assessment of the fetal heart between weeks 11 and 13.

2. Materials and Methods

Our retrospective study was approved by the Scientific and Investigational-Ethical Committee of the Hungarian Medical Scientific Council (2013/EKU (588/2013)). Data from the first-trimester fetal cardiovascular screening of consecutive patients from an unselected (or "routine") pregnant population at the RMC-Fetal Medicine Center throughout a study period from 1 January 2010 to 31 January 2015, were used to analyze the learning curve. Fetuses of pregnant women who received screening between 1 January 2011 and 30 June 2014, were monitored throughout pregnancy in order to assess the effectiveness of first-trimester cardiac screening.

Fetal cardiac scans were performed during routine first-trimester extended ultrasound screening, which included a comprehensive study of fetal sono-anatomy and aneuploidy, as well as preeclampsia screening in accordance with the protocol of the FMF (maternal age, body weight and height, maternal serum beta-hCG and PAPP-A assay combined with

fetal sonographic measurement of nuchal translucency (NT), nasal bone (NB) visualization, fetal heart rate (FHR) measurement, tricuspid regurgitation (TR), ductus venosus (DV) blood flow examination for aneuploidy, and maternal blood pressure, medical history, serum PAPP-A, and, as of 1 January 2014, serum PlGF assay combined with sonographic assessment of maternal left and right uterine artery Doppler-flow PI-value for preeclampsia screening, respectively) [33,35].

Transabdominal ultrasound examinations were conducted by using Accuvix V20 (Samsung-Medison, Seoul, Republic of Korea) and Voluson S8 (GE Medical Systems, Florecne, SC, USA) ultrasound scanners both equipped with 4 to 8 MHz convex abdominal transducers. At the beginning of the study period, ultrasound examinations were performed by three obstetricians (all of them were FMF-audited sonographers for NT, NB, DV, and TR), who were experienced in the assessment of normal fetal first- and second-trimester cardiac anatomy but only moderately experienced in the final evaluation of abnormal fetal cardiac anatomy. During the last year of the study period, an FMF-audited sonographer joined the obstetric team, who pre-screened pregnant patients.

Intrauterine cardiac screening was introduced in the 1980s, which at that time only consisted of the examination of 4 cavity planes, ideally at 18–22 weeks. Subsequently, it was recognized that in many cases, CHD exists despite the normal four-cavity view; therefore, by further increasing the detection rate, in 2001, Yagel et al., added the determination of the abdominal situs, aortic arch, ventricular outflow tract, and mediastinal vessel examination as part of the screening procedure. In 2015, the three-vessel tracheal view was incorporated into the UK screening protocol. During our screenings, we used all of the aforementioned techniques in the first-trimester heart examination [36–39].

In all cases, a comprehensive medical history was taken from the patients, including maternal body weight and height, temperature, and blood pressure. As of 1 January 2011, an institutional standardized protocol was introduced for sonographic examination of the first-trimester fetal heart, according to which the following planes, structures, and functions are to be examined and archived digitally:

1. Determination of abdominal situs;
2. Cardiac size and axis;
3. Four-chamber view: crux cordis, ventricular septum in the four-chamber-plane, ventricular color inflow, tricuspid pulsed-wave Doppler, and FHR;
4. Three-vessel view, V-confluence of color flow;
5. Longitudinal view: aortic arch with color flow, ductal arch, right ventricular outflow tract, imaging of the crossing of great vessels (during first-trimester cardiac screening it is more reliable to visualize a cross-section of the aorta next to the longitudinal section of the pulmonary trunk from a median-sagittal plane rather than using an axial cardiac grand-sweep);
6. Optional examinations of the left atrial pulmonary vein (at least two) and right atrial caval vein connections (veno-atrial concordancy).

Cardiac screening according to this protocol was considered comprehensive if all of the previously mentioned structures were stored digitally. No time limitations were applied during the examinations. The examination was only interrupted when a visualization obstacle other than fetal position occurred, such as a significant distance between fetus and transducer (retroflected uterus, thick maternal abdominal wall, previous abdominal scars, anterior placenta), and where vaginal ultrasound was also found to be inadequate [40,41]. In cases deemed to be within the normal range at first-trimester cardiac screening, if the patient returned to us later, the cardiac scan was repeated at 18 to 20 weeks and at 28 to 30 weeks. A pediatric cardiologist reevaluated all routinely screened cases that were found to be “abnormal”, as well as high-risk instances. The parents received thorough genetic counseling in the case that the prenatal cardiac screening revealed abnormalities.

Fetal karyotyping by chorionic villous sampling or amniocentesis was offered to all pregnant women who had positive first-trimester aneuploidy screening test results or if the type of CHD indicated it.

Pregnant women who underwent screening at our center received routine prenatal, genetic, and general obstetric care at various institutes of the country (221 attending physicians); therefore, data concerning the outcomes of pregnancies were collected from patients in three different methods:

1. Patients responded to our queries via email (our address appeared on the first-trimester obstetric ultrasound report);
2. Data were retrieved from the digital records of the obstetric division of our center where patients received prenatal care;
3. Verbal interviews with mothers by phone were performed by trained health personnel of our center. In the event of inconclusive outcome data, a follow-up interview via phone was repeated by the obstetrician to ascertain clear outcome results.

For statistical analysis, the Chi-Square Test was used and $p < 0.05$ was evaluated as a significant difference. The data were evaluated using GraphPad Prism 6.0. software.

3. Results

Overall, 42 (0.88%) cases of congenital heart disease were identified during the 4769 fetuses (155 twins, 6 triplets, and 4441 singletons) of 4602 pregnant mothers who underwent at least one first-trimester cardiac ultrasound screening between 1 January 2010, and 31 January 2015 (the majority also underwent second and third-trimester screening).

Table 1 presents the summary of the distribution of different types of CHD according to the timing of detection and gestational age.

Table 1. Prenatally observed abnormal hearts during the learning curve.

	2010	**2011**	**2012**	**2012**	**2014**	**Total**
# of 1st-trim. examinations	228	586	872	1228	1855	4769
# of abnormal hearts (1st-trim. Dx)	2 (1)	8 (7)	6 (5)	15 (14)	11 (9)	42 (36)
HLHS		2		1	1	4
AVSD	1	1	1	1	3	7
AVSD + HRH		1				1
AVSD + HLH			1		1	2
AVSD + Heterotaxia		1				1
VSD		1	2	3	1	7
VSD + LV < RV				1		1
ASD + LV < RV			1			1
LV > RV					1	1
LV < RV					1	1
HLH + VSD				1		1
HRH + VSD		1				1
Aortic stenosis + HLH + AVSD				1		1
Aortic atresia	1	1				2
Pulmonary valve regurgitation					1	1
Pulmonary atresia + HRH				1		1
Septal fibrosis + VSD				1		1
Ventricular fibro-elastosis				1		1
Rhabdomyoma					1	1
TGA					1	1
Tetralogy of Fallot				1		1
Isolated pericardial fluid			1	1		2
CHD—non evaluated				2		2

Abbreviations: #, number; Dx, diagnosis; HLHS, hypoplastic left heart syndrome; AVSD, atrio-ventricular septal defect; HRH, hypoplastic right heart; HLH, hypoplastic left heart (LV << RH); VSD, ventricular septal defect; LV, left ventricle; RV, right ventricle; ASD, atrial septal defect; TGA, transposition of great arteries; CHD, congenital heart defect.

Considering the low case number (n = 228) observed in the first study year (2010) and the absence of a uniformly applied examination protocol for the assessment of the fetal heart, fetuses screened during this particular time interval were not followed. In the group

of patients who underwent screening in the second half of 2014 (n = 1032 fetuses), the vast majority of pregnancies were still ongoing at the time of the outcome data analysis (from the 1 February 2015 to 31 March 2015). Consequently, only fetuses screened between the 1 January 2011 and the 30 June 2014 were followed and data retrieved from the outcome of the aforementioned fetuses were used to assess the effectiveness of screening.

During this three-and-a-half-year period, 3509 fetuses (125 twins, 6 triplets, and 3241 singletons) of 3372 pregnant women underwent a comprehensive fetal cardiovascular ultrasound screening at 11–13 + 6-weeks of gestation. The ratio of followed pregnancies was 93% (3142/3372). Of the 239 fetuses from 230 pregnancies who were lost at follow-up, no abnormalities were identified by a first-trimester fetal cardiac ultrasound scan. Of the 3270 followed fetuses, 3020 were singletons, 116 were twins, and 6 were triplets. The mean maternal age at the time of first-trimester screening was 33.9 years (range: 17–45). The ratio of pregnant women aged > 35 years was 46.9%. The mean fetal crown-rump length (CRL) was 64.0 mm (range: 45.0–84.0).

The mean maternal ages at the time of first-trimester screening in the entire study population and in the group of pregnancies affected with major fetal CHD were 33.9 and 35.8 years, respectively (nonsignificant, $\chi^2 = 3.601$, df = 1, $p = 0.058$). The proportions of women above 35 years of age in the overall study population and in the group of pregnancies affected by major fetal CHD were 46.9% and 71.0%, respectively (significant, $\chi^2 = 6.642$, df = 1, $p = 0.0099$).

The results of our NT, DV, and TR measurements (FMF software version 2.3) performed in conjunction with fetal cardiac screening revealed that 56.1% of NT values were above the median and 4.9% of them were above the 95th percentile, respectively. Simultaneously, in 54% of prenatally detected cardiac defects (20/37), NT-values were below the 99th percentile (<3.5 mm), and in 46% of them (17/37), the NT-value was less than 2.5 mm. With respect to other cardiac markers, TR was found in 0.6% of non-CHD cases and in 29% of fetuses with CHD (10/35) (significant, $\chi^2 = 308.486$, df = 1, $p < 0.05$. Similarly, an abnormal DV blood flow pattern was identified in 4.3% of all examined fetuses and in 51% of fetuses affected with cardiac anomalies (18/35) (significant, $\chi^2 = 168.282$, df = 1, $p < 0.05$).

3.1. Prenatal Diagnosis

During the first-trimester cardiac ultrasound screening of 3270 followed fetuses, 34 hearts were identified by the examining moderately experienced obstetricians as 'abnormal'. Of the cases, two were deemed to be within the normal range (#33 and 34), the initial diagnosis was either refined or supplemented in five(#4, 14, 21, 24, and 32), and three cases were not examined (#6, 15, and 18) by the pediatric cardiology specialist. In 24 cases, the cardiologist agreed with the initial diagnosis. In the three non-examined cases by pediatric cardiology specialists, pregnant women did not comply with repeated fetal cardiac ultrasound scans because, due to the presence of other associated fetal congenital malformations, they opted for pregnancy termination. Of the 32 prenatally verified abnormal fetal hearts (examined by both moderately experienced obstetricians and a pediatric cardiology specialist), 6 cases were interpreted as 'minor anomaly' (#3, 10, 28, 30, 31, and 32), while the remaining cases (n = 26) were evaluated as 'major cardiac vitium'. CHD were considered to be 'major anomalies' if they required cardiac surgery during the first year of life.

Two minor anomalies that were also confirmed by the cardiologist at the first-trimester scan (#31: isolated pericardial fluid; and #32: minor inlet ventricular septal defect, VSD), which were considered as a 'normal heart' at the second-trimester ultrasound screening. One case of fetal septal fibrotic area detected by both the obstetrician and the cardiologist at the time of first-trimester cardiac screening was supplemented with the diagnosis of VSD during second-trimester ultrasound screening (#28). Case #30 (isolated pericardial fluid at first-trimester screening), interpreted as a 'minor anomaly', proved postnatally to be a major cardiac defect (complex pulmonary atresia, Table 2).

Table 2. Abnormal cardiac findings during the following period.

	1st-Trim. OB. Dx	1st-Trim. Card. Dx	NT	TR	Abn. DV	20-Week OB + Card.	Assoc. Malformations	Karyotype	Pregnancy Outcome
1	AVSD	idem	2.3	+	+	-		47XX, +21	Termination
2	AVSD, HeterotaxiaDextrocardia	idem	1.7	not done	+	-	-	-	Termination
3	VSD	idem	7.9	+	+	-	-	-	Termination
4	AVSD + HRH	id. + fibro-elast	2.5	-	+	-	SUA	46XY	Termination
5	AVSD + HLHS	idem	1.9	-	-	-	-	46XX, 16 polymorph.	Termination
6	LV < RV, ASD	Not examined	5.1	-	-	-	Omphalocele paklatoschisis	47XX, +13	Termination
7	Tricu. atr, HRH, VSD	idem	1.6	Flow:-	-	-	-	46XX (abortum)	Termination
8	AVSD	idem	5	+	_	-	Oligohydramnios	-	Termination
9	HLHS	idem	6	+	-	-	Holoprosencephalia Polydactylia	47XY, +13	Termination
10	VSD (subaortic)	idem	N/A	not done	not done	-	Hydronephrosis Camptodactylia SUA oligohydramnion	69XXX	Termination
11	VSD, fibro-leastosis	idem	2.1	-	-	-	-	-	Termination
12	PA atr., HRH	idem	3.9	-	-	-	-	-	Termination
13	RV fibro-elastosis	idem	5.5	not done	not done	-	-	-	Termination
14	HLHS, VSD	HLHS, AVSD Aorta stenosis	1.5	+	not done	-	-	-	Termination
15	Left rot., Abn. GA	Not examined	6	not done	+	-	cervical cyst, short bones	PCR negative Cytogenetics cannot be performed:	Termination
16	HLHS	idem	2.4	-	+	-	Alob. holoprosencephaly Palatoschisis	-	Termination
17	HLHS, VSD,	idem	7	-	not done	-	SUA Holoprosencephaly Encephalocele Omphalocele	-	Termination
18	Abn. 4-CV + outfl. tr.	Not examined	8	not done	+	-	-	-	Termination
19	LV < RV, VSD	idem	7.3	-	-	-	Cleft lip and palate	47XX, +13	Termination
20	Tetralogy of Fallot	idem	4.5	.	+	-	-	-	Termination
21	LV < RV, AVSD	only AVSD	3.6	-	+	-	-	47XX, +21	Termination
22	AVSD	idem	4.7	+	+	-	Clubfoot, short bones	-	Termination
23	LV < RV,	idem	9	not done	+	-	Strawberry shape skull	-	Termination
24	RV > LV, P. valve reg.	P.valve reg.	1.8	+	+	-	-	47XX, +17 Chr 16 polymorph.	Termination
25	AVSD	idem	5.1	+	+	-	Short bones	-	Termination
26	AVSD	idem	6.5	+	+	-	-	-	Termination
27	HLHS	idem	1.2	not done	+	-	Omphalocele Cheilo-palatoschisis	-	Termination
28	Septal fibrotic area, Rhabdomyoma	idem	1.6	-	-	idem + VSD; Swiss cheese VSD	-	46XX	Born, idem, closed VSD No VSD Ventricular septum in upper third of echo-dense terime

Table 2. *Cont.*

	1st-Trim. OB. Dx	1st-Trim. Card. Dx	NT	TR	Abn. DV	20-Week OB + Card.	Assoc. Malformations	Karyotype	Pregnancy Outcome
29	HLHS	idem	3.1	+	+	-	-	47XX, +21	Termination
30	Isol. pericard. fluid	idem	2.1	-	-	hasn't come back	-	-	Born, complex P. atresia 1 year old with com-plex pulmonary atresia
31	Isol. pericard. fluid	idem	1.8	-	-	normal heart	-	-	Born, healthy
32	Large VSD	Min. inlet VSD	2.8	-	-	normal heart	-	46XX	Born, healthy
33	RV < LV	normal heart	4.8	+	-	normal heart		46XX, NT panel negative	Born, healthy
34	PA < Ao	normal heart	2.1	-	-	normal heart		-	Born, healthy
35	normal heart	-	2.4	-	-	VSD (subaortic)	Cleft lip	46XY	Born, VSD closed
36	normal heart	-	2.4	-	+	Ao. atr, LV > RV, fibr endocardialis fibrosis		46XY	Termination
37	normal heart	-	2	-	-	3-trim. Rhab-domyoma; Multiplex rhabdomy-oma	-	-	Born, Dx proved
38	normal heart	-	1.9	-	+	VSD (inlet)		46XY	Born, Dx proved
39	normal heart	-	3.3	-	-	small VSD	-	NIPT: negative	Born, cl. lip, VSD closed

Abbreviations: HLHS, hypoplastic left heart syndrome; AVSD, atrio-ventricular septal defect; HRH, hypoplastic right heart; HLH, hypoplastic left heart (LV << RH); VSD, ventricular septal defect; LV, left ventricle; RV, right ventricle; ASD, atrial septal defect; TGA, transposition of great arteries; CHD, congenital heart defect; Dx, diagnosis; OB, medium-experienced obstetrician; Card, pediatric cardiologist specialist; NT, nuchal translucency; TR, tricuspidal regurgitation, DV, ductus venosus; Abn, abnormal; Assoc, associated; Tricu atr, tricuspidal atresia; PA, pulmonary artery; Rot, rotation; GA, great arteries; 4-CV, four-chamber view; Outfl. tr., Outflow tracts; P. valve reg., Pulmonary valve regurgitation; Pericardial, pericardial; Ao, Aorta; idem (id), identical; N/A, non-available; SUA, single umbilical artery; Alob, alobar; cl. lip, cleft lip.

Of the 3238 first-trimester fetal hearts that were interpreted as 'normal' by moderately experienced obstetricians, second-trimester fetal echocardiography was indicated due to the presence of abnormal NT, DV, or TR, that verified three further minor anomalies (VSD; case #35, 38, and 39) and one major vitium (case #36: aortic stenosis + diffuse endocardial fibroelastosis). In one case, intensive fetal cardiologic observation and follow-up were indicated due to a positive family history; however, neither first- nor second-trimester screening found any abnormality. In the third trimester, however, multiple rhabdomyomas were detected (case #37).

3.2. Outcomes of Pregnancies

Of the 3270 followed fetuses, 3191 (97.6%) were born, while 79 fetuses (2.4%) were either not born or died early. Perinatal mortality was observed in 8 cases as a result of infection or for reasons that remain unclear. Major CHD was not detected in this cohort of patients. Spontaneous abortion occurred in 18 cases (0.5%) during the mid-trimester; there were no cases of major CHD in this group of patients, and first-trimester cardiac screening was negative for all of these cases. A total of 53 pregnancies (1.6%) were terminated for a genetic indication, of which 29 were found to have CHD (Table 3).

Table 3. Outcome of pregnancy in the follow-up period.

Number of Pregnancies Followed	n = 3270
Live births	3191 (97.6%)
Pregnancy losses	79 (2.4%)
Perinatal deaths (intrauterine demise + neonatal death)	8 (0.2%)

3.3. Postnatal Diagnoses

A total of 13 newly diagnosed minor cardiac anomalies were identified in the 3191 newborn infants (of which 10 cases of innocent cardiac murmurs and 2 cases of patent foramen ovale were excluded from the analysis as these are not considered cardiac anomalies, Table 4). In case #30, at one year of age, a pediatric cardiologic examination diagnosed a major cardiac malformation (complex pulmonary atresia, Table 3). In this particular case, the initial first-trimester cardiac scan showed a normal appearance of the four-chamber view and that of ventricular outflow tracts; however, the presence of isolated pericardial fluid was detected. Unfortunately, the pregnant patient did not attend the scheduled fetal echocardiography or the routine second and third-trimester obstetric ultrasound screenings.

Table 4. Postnatally recognized cardiac findings.

Type of Cardiac Anomaly	Number of Fetuses
Ventricular septal defect (2–5 mm)	8
Atrial septal defect II	3
Aortic valve stenosis min. grade	1
Bicuspidal aortic valve + PFO	1
Complex pulmonary atresia	1
TOTAL	14
(PFO + innocent cardiac murmurs)	(2 + 10)

Abbreviation: PFO: patent foramen ovale.

In case #32 (minor inlet VSD), second-trimester fetal echocardiography revealed that minor cardiac anomalies suspected prenatally at first-trimester screening were found to be negative, confirming a normal neonatal heart. In case #28, second-trimester fetal echocardiography augmented the diagnosis of first-trimester septal fibrotic area with VSD, and that finding was only supported partially after birth (the VSD was subsequently proved to be closed). In case #39, the initial first-trimester cardiac scan displayed a normal fetal heart, while abnormal NT was 3.3 mm, and non-invasive prenatal testing (NIPT) was negative. However, second-trimester fetal echocardiography detected a small VSD that was confirmed to be closed after birth. In the other two minor fetal cardiac anomalies diagnosed at the second-trimester fetal echocardiography, postnatal cardiac evaluation confirmed the prenatal diagnoses.

In summary, 49 (1.49%) cardiac abnormalities were observed in the 3270 fetuses followed. Thirteen new cardiac abnormalities (28.6%) were first discovered postnatally (1 major and 13 minor defects), while 35 (71.4%) of the 49 cardiac anomalies were diagnosed prenatally (27 major and 8 minor defects).

Of the four small VSDs diagnosed prenatally, only two cases were confirmed postnatally, supporting the possibility of spontaneous intrauterine closure (healing) of minor fetal cardiac septal defects.

Of the fetuses followed, 96.4% of major CHD (27 of 28) were diagnosed prenatally by moderately experienced obstetricians in collaboration with a pediatric cardiology specialist, and 89.2% of them (25 of 28) were already diagnosed (or at least highly suspected) at first-trimester screening and subsequent fetal echocardiography.

In the first year of the five-year study period (2010), our objective was to visualize and store as many clear planes of the first-trimester fetal heart as possible; therefore, our reports

were limited to fragments of a complete cardiac examination panel, including only a true four-chamber view plane. Of the 282 first-trimester cardiac scans conducted, only one abnormal fetal heart with an atrio-ventricular septal defect (AVSD) and one case of aortic stenosis were diagnosed in the second trimester (Table 1). During the 2011–2012 (already followed) study period, 11 major cardiac defects were identified from a significantly larger group of examinations (n = 1458), and 9 of them were picked up at the first-trimester scan. In one of the two cases where CHD was not identified (case #36, aortic atresia, left ventricle >> right ventricle, fibrosis, see Table 2), the four-chamber view (at later offline analysis the aorta appeared slightly narrower) at first-trimester scan appeared to be normal and the patient had a high risk for trisomy 21 (1:4); both were both ruled out at subsequent chorionic villous sampling (CVS). At the 18-week scan, a definitive diagnosis of fetal aortic atresia was established. The second undiagnosed cardiac vitium (detailed in the 'results' section) was only detected after birth (complex pulmonary atresia, case #36, see Table 2).

Of the 9 diagnosed first-trimester major cardiac defects, detailed fetal echocardiography led to the diagnosis of Down's syndrome in two cases (case #1: AVSD and case #29: hypoplastic left heart syndrome, HLHS). In cases #2, 4, 5, and 7, fetal cardiac anomalies were identified with an NT value within the normal range and the karyotype was also normal. In one case (case #2), assessment of the situs and the four-chamber view led us to the final diagnosis of AVSD combined with heterotaxy (see Table 2). During the second half of the study (2013–2014), all major cardiac defects were identified at first-trimester screening, with the exception of one case of fetal rhabdomyoma (Table 1).

4. Discussion

The clinical data of unselected consecutive pregnant women screened at our center indicate that the examined population should be considered to have an intermediate-risk profile with respect to maternal age. No statistically significant difference was observed in terms of maternal age between the entire study population and the group of pregnancies affected by major fetal CHD. In contrast, the incidence of major fetal CHD was significantly higher for the subset of women aged > 35 years.

This phenomenon highlights the increased incidence of congenital malformations, including CHD associated with advanced maternal age and chromosomal abnormalities [42,43]. The present study demonstrated that an abnormal fetal karyotype was present in 24% of cases (9/37). It is important to note that older maternal age is a significant contributing factor to fetal heart development defects. Both fetal and maternal complications increase significantly with a maternal age of 35 years during pregnancy. A maternal age of greater than 35 years has recently been associated with subclinical myocardial dysfunction [44] and several obstetrical complications (gestational diabetes, gestational hypertension, pre-eclampsia) [45–47].

On the other hand, a typical tendency in Hungary over the last decade is that older pregnant women with better socio-economic status prefer to undergo high-standard screening and diagnostic obstetric examinations in well-equipped specialized private obstetric clinics (with well-trained specialists) leading to a very high detection rate of various fetal pathologies. Last but not least, primary care obstetricians tend to refer their high-risk patients immediately and directly to these perinatal centers.

According to the meta-analysis, the detection rate of congenital cardiac defects increases by up to 23% [28,48]. The newly formed high-risk group of routinely screened fetuses with NT-values above the 99th percentile (cut-off > 3.5 mm) was detected. Many studies have proved that other markers for first-trimester screening for aneuploidies, such as TR and DV, are useful for screening for congenital cardiac defects. The combination of NT measurement with TR and DV blood flow assessment reaches a detection rate of 48% for major congenital cardiac defects in the first trimester [49,50].

The NT, DV, and TR measurements of our study support the data that these novel cardiac markers (NT, TR, and DV), introduced in extended first-trimester screening, can significantly narrow and refine the cohort of pregnant patients at risk for CHD beyond

those at high baseline risk with classic indications. Nevertheless, the routine clinical use of NT, DV, and TR alone will not result in the detection of the vast majority of congenital cardiac defects [28,48,50,51]. To achieve this, it is essential that a structural evaluation of the fetal heart be performed in both maternal age-related and abnormal NT-, TR-, and DV-related risk pregnancy groups, as well as in the group of pregnant women at high baseline risk for CHD with classic indications.

The examinations were conducted using state-of-the-art ultrasound equipment; however, instead of high-frequency linear probes designed specifically for cardiac scanning, we used medium-frequency and resolution convex abdominal transducers with wide view-angle and greater tissue depth penetration, suitable for complete first-trimester ultrasound screening. Due to the limited maneuverability of transvaginal ultrasound probes, this approach was rarely used, mostly in cases with a retroflected uterus. Our experience supports the observation that optimal visualization is not dependent solely on body mass index (BMI) but rather on transducer-fetal heart distance, which is influenced by BMI, uterine, placental, and fetal position [28,31,32,34,52]. The extended first-trimester screening examinations were performed by three obstetricians, who had 4 to 8 years of mid-level sonographic experience in fetal diagnostics (mainly second-trimester), one-year FMF-TR/DV audit at the beginning of the study (2010), and little experience in assessing healthy and abnormal fetal heart. At the beginning of the study period, inexperience was offset by the time dedicated to the examination; some cases were analyzed offline for 3 to 4 h. Later on, a highly-qualified sonographer joined our team and her excellent overall performance in the pre-screening allowed the obstetricians to focus on the evaluation of pathologic findings and, subsequently, the total examination time was reduced to 20–30 min. A pre-screening ultrasound by a sonographer should always be followed by post-screening performed by an obstetrician preferably using check-lists to ensure continuous quality control.

Previously, our pediatric cardiologist was used to performing echos on fetuses over 16 weeks, but thanks to her openness, she became more and more comfortable with first-trimester ultrasounds. By the end of the study period, fetal hearts interpreted as 'abnormal' by moderately experienced obstetricians were rescanned by the pediatric cardiologist and pregnant mothers received a detailed fetal cardiologic second opinion within 24 h, significantly reducing their psychological distress.

Case #36 supports the novel findings that the progression of CHD may be intense, especially up to the 20th week of pregnancy.

In the initial period of the learning curve (2010–2012), the typical and common features of the nine cases of CHD detected in the first trimester were an abnormal four-chamber view and failure to demonstrate the involvement of the great vessels in the cardiac defect. (A limitation of each study, including ours, which addresses the prenatal sonographic assessment of abnormal hearts, is that subsequent histopathological evaluation of the first-trimester fetal heart is not feasible in the majority of cases). Although the assessment of outflow tracts did not contribute to the diagnosis of CHD, we believe that it was nevertheless an invaluable experience. The long timeframe available for extended first-trimester screening resulted in significantly enhanced proficiency in delicate transducer movements and empirical learning.

Table 1 shows that during the second half (2013–2014) of our study, first-trimester abnormal great-vessel diagnoses were also observed (Tetralogy of Fallot, Transposition of the Great Arteries).

5. Conclusions

According to our research, if appropriate equipment, quality control, and motivation are in place, the effectiveness of first-trimester fetal cardiovascular ultrasound screening by moderately experienced obstetricians in an unselected (so-called "routine") pregnant population may reach 90% in terms of major congenital heart defects.

Our data demonstrate that early fetal echocardiography not only provides reassurance to the majority of pregnant women with regard to the absence of cardiac anomalies, as

the most common congenital birth defect, but also significantly contributes to the early diagnosis of chromosomal abnormalities. The majority of cases in which fetal cardiac anomalies were detected are usually so complex and severe that they allow couples to opt for an early termination of their pregnancies and thus decrease maternal general medical risks and the psychological burden of second-trimester elective termination.

The use of first-trimester cardiac markers (NT, TR, and DV) may draw our attention to serious complex structural heart defects and may also help in the detection of minor and major fetal cardiac anomalies that are only visualized at second-trimester screening. Subsequently, it may provide an opportunity to improve neonatal morbidity and mortality as a result of prior organization of intensive perinatal cardiac care.

Second-opinion fetal echocardiography, performed by pediatric cardiology specialists to re-assess suspected cardiac anomalies by moderately experienced obstetricians, is crucial as correction and/or augmentation of established diagnoses will help genetic counselors and couples to better understand the severity, complexity, and prognosis of the condition.

The immediate availability of a pediatric cardiologist can significantly reduce the psychological stress that parents experience as a result of the diagnostic uncertainty of the obstetricians.

In our study, we presented a 5-year learning curve, at the conclusion of which we had performed a high case number of nearly 4800 first-trimester fetal cardiac scans, identified over 40 fetal cardiac anomalies, and significantly enhanced proficiency among physicians in visualizing and assessing both four-chamber view- and outflow tract-planes, as well as large vessel relations of the 11 to 13-week fetal heart.

It may be reasonably assumed that an experienced obstetrician-sonographer performs at least 1300 first-trimester aneuploidy and cardiovascular screens annually. Consequently, in a country like Hungary, only 10–15% of moderately and highly experienced obstetrician-sonographers would require specialized training to enable them to perform detailed early fetal echocardiography, thereby ensuring that every pregnant Hungarian woman has access to this service. Provided that a structured high-quality training program is made available, this could be achieved in a shorter time span than our learning curve, and thus first-trimester fetal echocardiography might become part of routine first-trimester screening.

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Case Report

Second Trimester Ultrasound Diagnosis of External Hydrocephalus in Two Fetuses with Noonan Syndrome—Case Report Series

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Abstract: Background: Noonan syndrome (NS) is a relatively common RASopathy that can be associated with a variety of phenotypic and genotypic variations and potential long-term health consequences. Its most described prenatal ultrasound features in the first trimester are thickened nuchal translucency (NT) and dilated jugular sacs; while heart defects, polyhydramnios and facial dysmorphisms are its known manifestations in the second and third trimesters. **Methods:** We present two cases of NS with the prenatal ultrasound diagnosis of external hydrocephalus (EH) in the second trimester. **Results:** Case 1 had a normal first trimester scan and showed mild polyhydramnios, an echogenic intracardiac focus (EIF) in the left ventricle and pyelectasis in the second trimester in association with the EH. The whole exome sequencing (WES) confirmed a pathogenic variant in the *SOS1* gene. Case 2 showed increased NT, agenesis of the ductus venosus (DV), single umbilical artery (SUA), an EIF in the right ventricle and an abnormal prefrontal space ratio (PSFR). By the 19th gestational week, EH appeared. The ambient and quadrigeminal cisterns were also slightly widened. The WES revealed a *PTPN11* gene variant. **Conclusions:** The most reported sonographic features of NS are either non-specific or difficult to integrate into routine screening, requiring substantial experience. In our two cases, we detected EH in the second trimester, which is rarely described as a prenatal ultrasound diagnosis. To our current knowledge, this is the first case reported of EH in NS caused by an *SOS1* gene variant and these are the first cases reported with the prenatal sonographic diagnosis of EH in NS.

Keywords: Noonan syndrome; external hydrocephalus; subarachnoid space

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1. Introduction

Noonan syndrome is a relatively common RASopathy associated with a variety of phenotypic and genotypic features. Its prevalence is estimated to be 1:1000–2500, but in mild cases might be higher [1,2] and, therefore, more common than Edwards or Patau syndrome [3]. It is named after Jacqueline Noonan, an American pediatric cardiologist who published a paper in 1963 describing nine patients with pulmonary valve stenosis and various other coexisting conditions [4].

NS is mainly inherited in an autosomal dominant manner and in about 50% of the cases due to a variant in the *PTPN11* gene (176876). Other known gene variations that lead to NS are *NS3* (609942), *KRAS* (190070); *NS4* (610733), *SOS1* (182530); *NS5* (611553), *RAF1* (164760); *NS6* (613224), *NRAS* (164790); *NS7* (613706), *BRAF* (164757); *NS8* (615355), *RIT1* (609591); *NS9* (616559), *SOS2* (601247); *NS10* (616564), *LZTR1* (600574); *NS11* (618499), *MRAS* (608435), *NS12* (618624), *RRAS2* (600098), *NS13* (619087) and *MAPK1* (176948), while the autosomal recessive form of Noonan syndrome is caused by *NS2* (605275), *LZTR1* (600574), *NS14* (619745) and *SPRED2* (609292) [5–7].

Noonan syndrome is characterized by typical facial dysmorphism, skeletal anomalies, webbed neck and congenital heart defects [1].

Following Down syndrome, Noonan syndrome is the second most common genetic disorder associated with heart defects, typically with pulmonary stenosis and hypertrophic cardiomyopathy [8].

The prenatal diagnosis of Noonan syndrome is often challenging due to variability in the phenotypic presentation and occasionally mild and non-specific signs [9,10]. In NS, the most frequently reported prenatal ultrasound findings are increased NT, cystic hygroma, dilated jugular lymphatic sacs, thickened nuchal fold, cardiac defects, kidney malformations and polyhydramnios [9,11–13].

In those cases in which the fetus does not show the typical signs associated with Noonan syndrome, the detection rate remains low and, due to the often non-specific findings, the diagnosis might be delayed or missed even in postnatal life [9,14].

External hydrocephalus is defined as enlarged subarachnoid spaces detected on imaging modalities with normal or mildly dilated ventricles [15]. In the presented cases, the authors used the depth of the Sylvian fissure to determine the width of the subarachnoid spaces, based on the normal value database published by Alonso et al. [16].

In this paper, we report two cases of prenatally confirmed Noonan syndrome showing the rare sonographic finding of external hydrocephalus in the second trimester.

2. Case Report Series

We present two cases of Noonan syndrome with external hydrocephalus in the second trimester in the presence of the variations of the *SOS1* and *PTPN11* genes. To our current knowledge, this is the first paper describing the association of prenatal external hydrocephalus in Noonan syndrome caused by a variation of the *SOS1* gene, and these are the first cases reported with a second trimester sonographic diagnosis of EH in NS. This case report is structured according to the CARE guidelines. We defined external hydrocephalus on the level of Sylvian fissure: measure over 95 percentile was defined as EH.

2.1. Case 1

2.1.1. Patient Information and Obstetric History

The 46-year-old pregnant woman first presented for the first trimester screening at the gestational age of 12 weeks and 5 days. The mode of conception was IVF from oocyte donation (donor is under 25 years of age). She previously had a missed abortion at the 7th gestational week.

She had a medical history of antiphospholipid syndrome, hypothyroidism and insulin resistance, her regular medications were metformin, levothyroxine, liothyronine, progesterone, escitalopram in combination with clonazepam, and enoxaparin. No family history of genetic disease was known. Body mass index (BMI) was in the normal range.

2.1.2. Ultrasound Findings

The first trimester extended ultrasound scan (NT, nasal bone, DV, tricuspid valve regurgitation, early cardiac and intracranial scan) revealed no anomalies. The thickness of the nuchal translucency was 2.4 mm, representing 85 percentile based on the measured CRL of 69.9 mm, which was 3 days greater than the calculated gestational age according to the IVF.

In the second trimester, a mild polyhydramnios was found (deepest vertical pocket: 9.3 cm, amniotic fluid index: 21 cm). BPD (52.2 mm) and HC (191.1 mm) were above the 90th percentile, all other biometric data were in the normal range. The diameter of the posterior horn at the atrium of the lateral ventricle was 9 mm. The ambient and quadrigeminal cisterns appeared slightly dilated (Figure 1), and the Sylvian fissure measured 10.5 mm, which was above the 95th percentile (Figure 2a). In addition, a mild hypertelorism (outer-to-outer distance: 35 mm, inner-to-inner distance: 16 mm), an echogenic intracardiac focus (EIF) in the left ventricle and mild bilateral pyelectasis were detected. An echocardiogram was performed by a pediatric cardiologist, which revealed no cardiac anomalies.

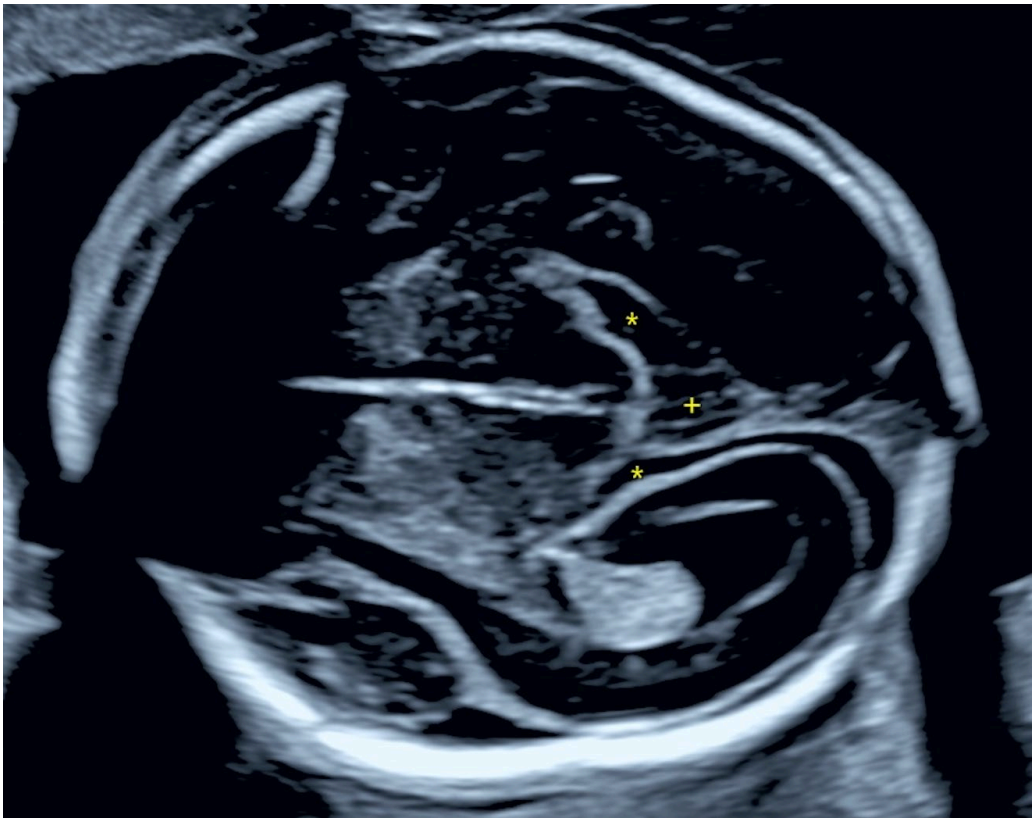
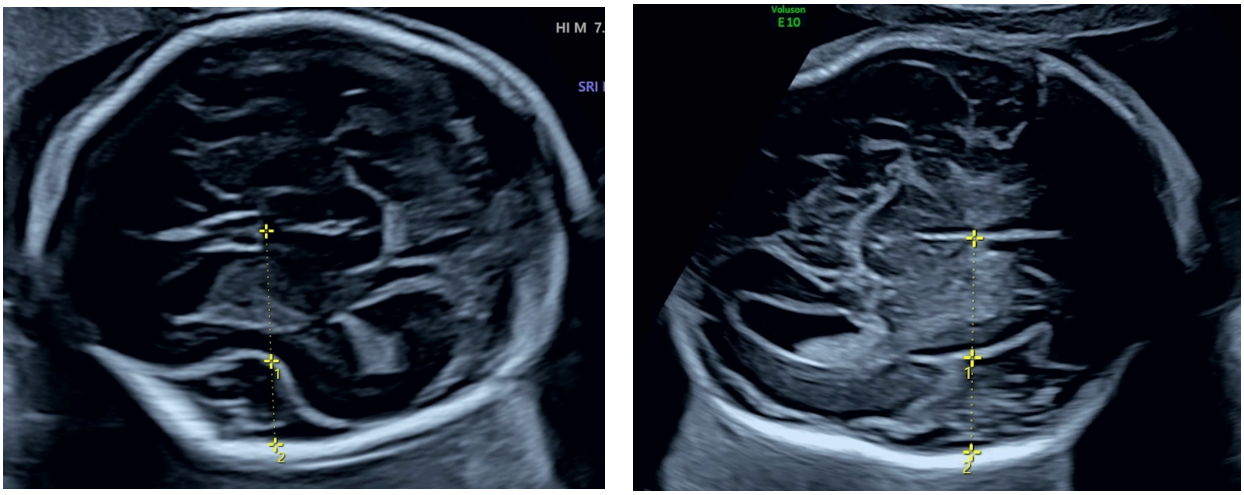


Figure 1. The enlarged ambient cisterns (*) and quadrigeminal cistern (+) at the 20th gestational week.

Fetal ultrasound follow-ups were performed at the 27th, 31st and 34th gestational week. Mild-to-moderate polyhydramnios was confirmed throughout the pregnancy. BPD and HC increased steadily, with both above the 99th percentile by the 27th gestational week. The ambient and quadrigeminal cisterns remained enlarged and the subarachnoid spaces widened. At the 27th gestational week, the depth of the Sylvian fissure measured 17.4 mm, which is high above the 95th percentile (Figure 2b) and the external hydrocephalus became evident also in the cranial views (Figure 3).



(**a**) (**b**)

Figure 2. Measurements of the Sylvian fissure. Measurements marked with 1 indicate the insular depth, measurements marked with 2 indicate the Sylvian fissure depth. (**a**) The depth of the Sylvian fissure was measured 10.0 mm at the 20th week; (**b**) and 17.4 mm at the 27th week. Both measurements exceeded the 95th percentile according to the database published by Alonso et al. in 2010 [16].

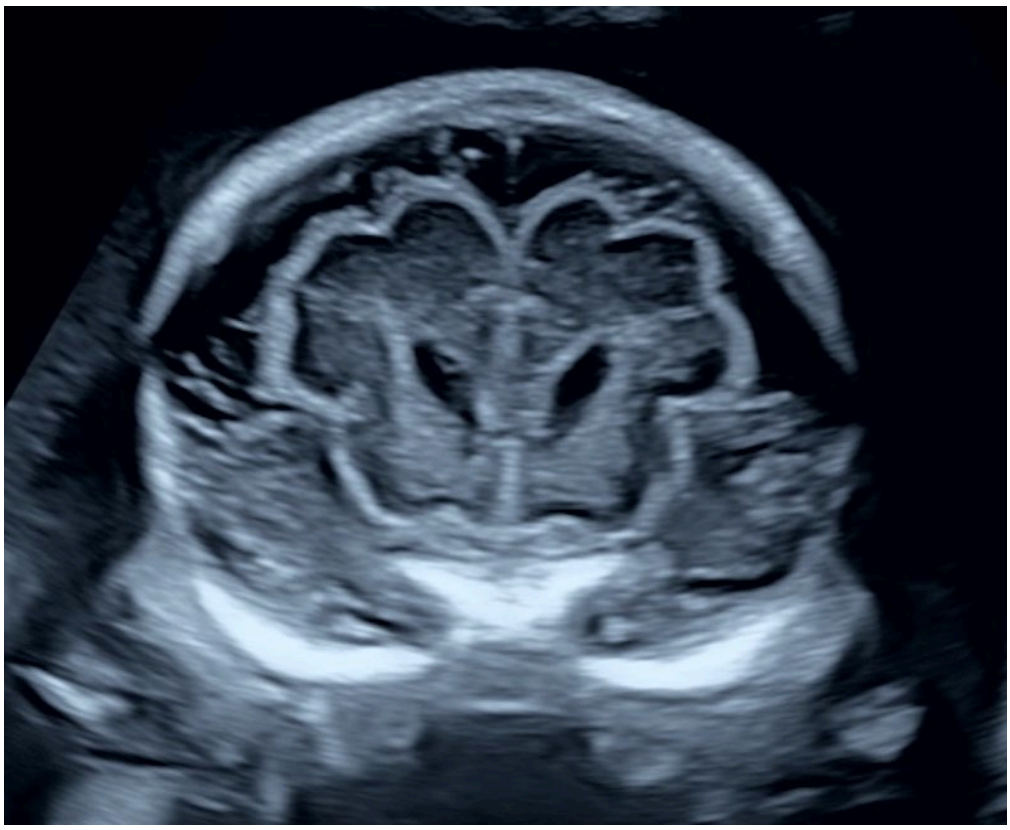


Figure 3. Coronal transcaudate plane in the 27th gestational week. The dilation of the subarachnoid spaces became obvious.

AC was measured at the 98th percentile at the 27th week, with an estimated fetal weight at the 97.9th percentile. These biometric data were maintained throughout the pregnancy. A mild dilatation of the right kidney remained with a diameter of 8 mm measured at the 34th week of pregnancy, while the width of the right kidney pelvis was normalized.

2.1.3. Genetic Studies

A cf-DNA test was performed in the first trimester with normal results. An amniocentesis was performed following the second trimester scan. No chromosomal anomalies were confirmed by G-band analysis of the amniotic cell culture (46XY). The array CGH confirmed a 2 Kb heterozygous microdeletion affecting the *RAB6C* (OMIM: 612909) and *SMPD4* (OMIM: 610457) genes. The following whole exome sequencing ruled out any compound heterozygosity and revealed a heterozygous pathogenic variation in the *SOS1* gene (NM_005633.4:c.1644T>A, p.Ser548Arg) that confirmed Noonan syndrome 4 (Phenotype MIM number: 610733, location: 2p22.1, Gene/Locus MIM number: 182530).

2.1.4. Perinatal and Postnatal Outcome

After detailed genetic counselling, the pregnant woman decided to proceed with the pregnancy. The baby was born by caesarean section in the 38th gestational week, with a birth weight of 3530 g, Apgar 9/10. The routine neonatal screening tests for vision and hearing were normal.

The newborn underwent regular cardiological follow-up, which revealed mild supravalvular pulmonary stenosis with no progression during the follow-up period to date. The last check-up was at age 2, requiring no intervention and annual follow-ups.

A cranial ultrasound at 7 weeks of age did not report any anomalies. The neurodevelopmental examination revealed minimal whole-body hypotonia and moderately delayed psychomotor development. Complex (motor, mental, speech) rehabilitation was recommended with a follow-up examination in 1 year.

2.2. Case 2

2.2.1. Patient Information and Obstetric History

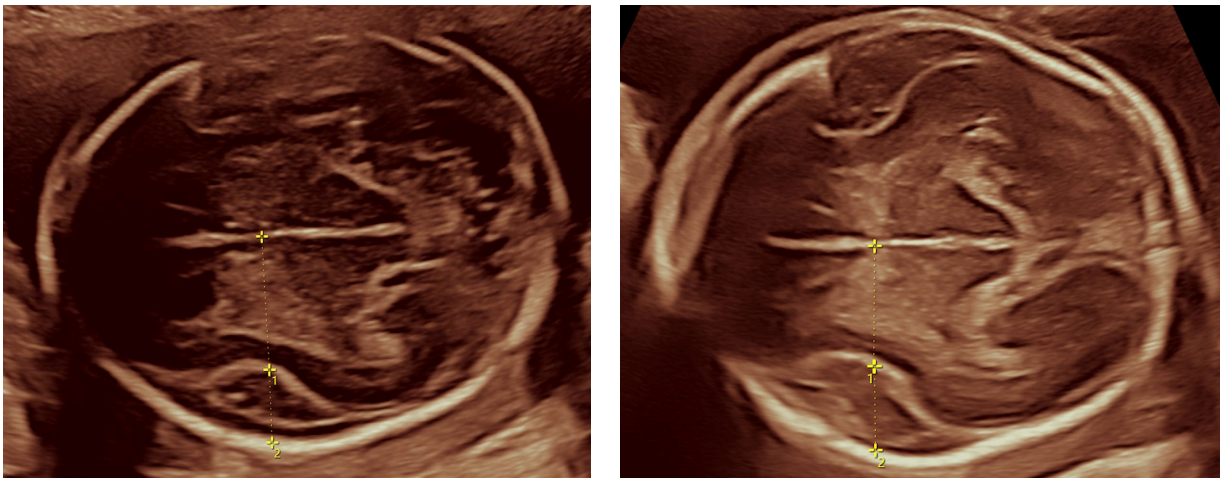
The 32-year-old pregnant woman was referred to our clinic with her spontaneously conceived second pregnancy. She had a previous normal vaginal delivery at the 39th gestational week following an uneventful pregnancy. Her only chronic medical condition was hypothyroidism that required no treatment. No family history of hereditary disease was known and BMI was in the normal range.

2.2.2. Ultrasound Findings

The first trimester screening was performed in another clinic, NT was measured 3.2 mm and an agenesis of the DV was suspected; therefore, she was referred to our center.

We confirmed an NT of 3.4 mm, representing 99 percentile according to the CRL of 70.6 mm, a DV agenesis, an SUA, an EIF in the right ventricle and frontally depressed cranial bones. A follow-up ultrasound scan at the 16th gestational week confirmed these anomalies and the DV agenesis (intrahepatic portosystemic shunt). A fetal echocardiogram performed by a pediatric cardiologist showed no evidence of major cardiac malformation; however, a small subaortic ventricular septal defect (VSD) with a suspected septal membrane aneurysm was reported. At the 21st week, in addition to the previously described anomalies, an abnormal PFSR and heart ventricular hypertrophy were confirmed.

At the 19th week, a dilated Sylvian fissure of 8.0 mm was found along with an otherwise normal neurosonography. At the 22nd gestational week, the depth of the Sylvian fissure was measured 10.8 mm (Figure 4). Both of these measurements represent values above the 95th percentile. The expansion of the subarachnoid spaces and Sylvian fissures was also remarkable in the coronal plane (Figure 5).



(**a**) (**b**)

Figure 4. Measurements of the Sylvian fissure. Measurements marked with 1 indicate the insular depth, measurements marked with 2 indicate the Sylvian fissure depth. (**a**) The depth of the Sylvian fissure was measured 8.0 mm at the 19th week; (**b**) and 10.9 mm at the 22nd week. Both above the 95th percentile according to the database published by Alonso et al. in 2010 [16].

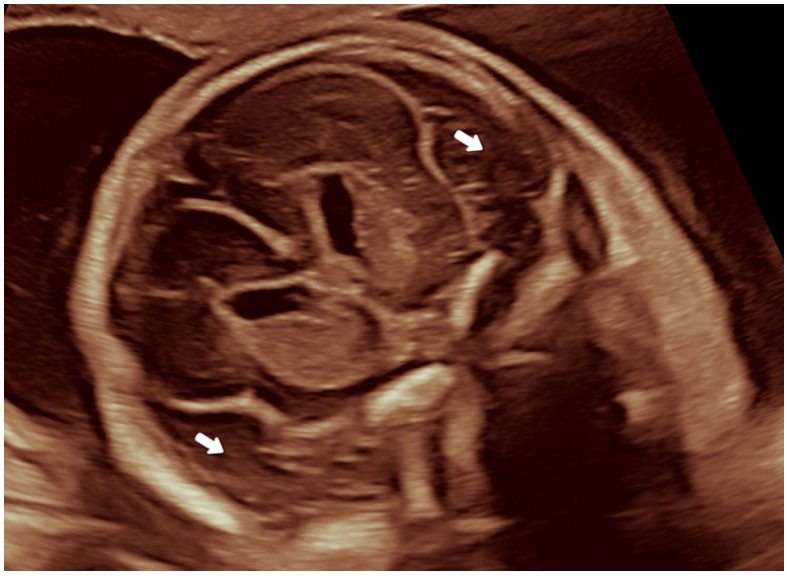


Figure 5. Coronal transcaudate plane in the 22nd gestational week. The dilation of the subarachnoid spaces and Sylvian fissures is notable (arrows).

2.2.3. Genetic Studies

No cf-DNA test was performed.

An amniocentesis was performed, and the results of the karyotyping and the SNP microarray revealed no pathologic findings. The whole exome sequencing found a heterozygous variation in the *PTPN11* gene (c.124A>G, p.Thr42Ala; chr12-112884189 A>G, NM_002834.5, rs397507501), confirming Noonan syndrome 1 (Phenotype MIM number: 163950, Location 12q24.13, Gene/Locus MIM number 1768762.2.4).

2.2.4. Perinatal and Postnatal Outcome

Following detailed genetic counseling and receiving comprehensive information, the pregnant woman decided to terminate her pregnancy.

3. Discussion

NS is a common genetic disorder, with a high variability in both genotype and phenotype. Due to the diversity of the syndrome, prenatal and even postnatal detection is often challenging [17].

Although NS can be detected by various genetic testing modalities such as targeted Noonan panels, WES and even cf-DNA tests, screening tests are not available routinely in all countries and might be a significant financial burden for patients, while diagnostic tests are carried out only based on a proper indication. Therefore, sonographic markers that could raise suspicion of this condition are playing an important role in providing effective prenatal screening and improving the detection rate [17].

Noonan syndrome is suspected mainly due to increased NT in the first trimester [9,11]. However, access to first trimester screenings might be influenced by the patient's socioeconomic status and personal preferences [18–20]. In a review published in 2024, Tangshewinsirikul et al. found increased NT to be the most frequently detected prenatal ultrasound anomaly in 105 cases of Noonan syndrome, occurring in 71% of the cases, followed by various body fluid collections (59%) and polyhydramnios (50%) [21]. Although in a study of 151 children with NS, only 12.5% of the echocardiograms were normal and 62% of the abnormalities were pulmonary stenosis. In the 105 fetuses with NS, the most commonly reported ventricular hypertrophy was found in 33% of the fetuses and pulmonary stenosis in only 13% [21,22].

The intracranial manifestations of NS have been described mainly in children or young adults and include hydrocephalus, Chiari I malformation, diverticular enlargement of the foramen of Luschka and cerebrovascular aneurysm [23–25]. Helenius et al. reported a 22-week-old fetus with enlarged extracerebral CSF and delayed operculisation in addition to a hypoplastic vermis on prenatal MRI in a case of NS with *RAF1* variation [26]. A widening of the subarachnoid spaces was described by Gripp in children with *SHOC2* and Zarate in a case with the *RAF1* variants, along with other extra- and intracranial abnormalities in individuals affected by NS. Mastromoro et al. described an MRI diagnosis of EH in the 27th gestational week following the genetic diagnosis of NS caused by a variation in the *PTPN11* gene. In their case, the corpus callosum and the cerebellar vermis were measured under the 10th percentile [27].

The prenatal MRI diagnosis of EH has previously been described in some disorders such as Snijders Blok–Campeau Syndrome and benign external hydrocephalus [28,29]. Baron et al. reported 21 cases of macrocephaly diagnosed by ultrasound, all of which had a fetal MRI, which confirmed the presence of EH in 77% of the cases [30]. In postnatal life, the enlargement of the subarachnoid fluid spaces was reported in connection with RASopathies [31].

A postnatally described central nervous system anomaly in the presence of the *SOS1* gene variation was a corpus callosum agenesis with severe developmental delay [32]. Another case report described spinal lesions resembling plexiform neurofibromas in NS caused by a variation in the *SOS1* gene [33].

The involvement of the two gene variants we identified, *SOS1* and *PTPN11*, in the development of the central nervous system has been previously reported. The *PTPN11* gene encodes the Shp2 protein and in NS it has a gain-of-function variation. In mice, Shp2 reduces the axon myelination and increases the density of the neurons but decreases the astrocyte density within the forebrain and the hippocampus. *SOS1* stimulates the nerve growth factor, which is expressed in the cortex of the newborns, and activates the Ras-MAPK pathway NMDA receptors in the neonatal cortex [34,35]. Whether these genes play a role in the development of EH in NS, and if so, via what exact pathway, requires further research.

In our two cases, EH occurred in the second trimester without any other significant central nervous system (CNS) anomaly and was confirmed ultrasonographically by measuring the depth of the Sylvian fissure in the axial plane. To our present knowledge, Case 1 is the first case of external hydrocephalus with an otherwise normal CNS in the presence of the *SOS1* gene variation. Furthermore, these are the first cases of NS with a second trimester ultrasound diagnosis of EH.

Neurosonography and MRI are complementary modalities in fetal diagnostics. According to the ISUOG guidelines, a fetal MRI is indicated in 7–15% of cases following a well-performed neurosonography [36]. In our experience, ultrasound examination alone was adequate for evaluating the subarachnoid spaces, offering better cost-effectiveness, faster diagnosis and lower human resource requirements compared to MRI. Despite the two cases presented, it must also be taken into account that EH may have developed independently of NS in both cases. However, since external hydrocephalus is a known phenomenon in children suffering from NS, it is reasonable to assume that the prenatal finding is related to the syndrome [26,37,38].

Examination of the subarachnoid spaces is currently not part of routine screening, and this may presumably contribute to undetected cases of external hydrocephalus. In the authors' opinion, the measurement of the depth of the Sylvian fissure may be a suitable tool for a second trimester prenatal diagnosis of external hydrocephalus, and as an additional marker, could contribute to the timely implementation of genetic testing, which can be beneficial in some regions due to the legal regulation of termination. The application of Sylvian fissure depth measurement in this context requires further studies, but as its technical performance does not require the acquisition of new skills, it could be easily integrated into routine screening.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author due to privacy reasons.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

AC	Abdominal circumference
BMI	Body mass index
BPD	Biparietal diameter
cf-DNA	Cell-free DNA
CNS	Central nervous system
CRL	Crown-rump length
DV	Ductus venosus
DVP	Deepest (maximal) vertical pocket
EH	External hydrocephalus

EIF	Echogenic intracardiac focus
HC	Head circumference
IVF	In vitro fertilization
NS	Noonan syndrome
NT	Nuchal translucency
PFSR	Prefrontal space ratio
SUA	Single umbilical artery
VSD	Ventricular septal defect

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Az első hazai tapasztalatok összegzése kromoszomális microarray-analízis és teljesexom-szekvenálás módszerekkel a magzati diagnosztikában

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Lukács Valéria dr.[7] ▪ Klujber Valéria dr.[8] ▪ Török Olga dr.[9]
Kiss Zsuzsanna dr.[10] ▪ Tardy Erika dr.[11] ▪ Tidrenczel Zsolt dr.[11]
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Az *Orvosi Hetilap* Szerkesztősége felkérésére készített tanulmányukat a Szerzők *Korányi Sándor* (1866–1944) belgyógyászprofesszor, a 20. század első felének kiemelkedő orvosegyénisége emlékének ajánlják halálának 80. évfordulóján.

Bevezetés: Az elmúlt évtized egyik jelentős technológiai újdonsága az ún. 'high-throughput' molekuláris genetikai vizsgálati módszerek – mint a kromoszomális microarray-analízis (chromosomal microarray analysis, CMA) és a teljesexom-szekvenálás (whole-exome sequencing, WES) – elterjedése a praenatalis diagnosztikában.
Célkitűzés: Az elmúlt 5 évben munkacsoportunk több mint 252 praenatalis vizsgálatot végzett hazai laboratóriumi háttérrel, amelyek indikációját különböző súlyosságú strukturális magzati ultrahangeltérések képezték. A klasszikus citogenetikai vizsgálatok eredményétől függően végeztük el a nagy felbontású CMA- és WES-analíziseket a praenatalis diagnosztika érdekében.
Módszer: A CMA-vizsgálatokat a „GeneChip System 3000 Instrument" platformmal végeztük az SNP-alapú komparatív hibridizálás módszerével. Az általunk elvégzett újgenerációs szekvenálás során a teljes humán exom szekvenciájának meghatározása IonTorrent és Illumina platformokkal történt.
Eredmények: Összesen 252 magzati CMA-vizsgálatot végeztünk, és 42%-ban mutattunk ki valamilyen hiányt vagy többletet, ebből 22%-ban igazoltunk kóros eltérést. 42 esetben végeztünk WES-t, amelyből 9 esetben (21,4%) azonosítottunk kóros eltérést az öröklésmenetet támogató, a magzati fenotípussal feltételezhetően összefüggésben lévő, a ClinVar adatbázis vagy az ACMG-klasszifikáció alapján.

*A szerzők egyenlő mértékben járultak hozzá a kézirat elkészítéséhez.
**A szerzők utolsó szerzőként egyenlő mértékben járultak hozzá a kézirat elkészítéséhez.

Megbeszélés: Tekintettel arra, hogy a magzati fenotípus értékelése közvetett, a praenatalis CMA- és WES-elemzésnek elsősorban a magzati ultrahangvizsgálat során azonosítható strukturális anomáliákkal összefüggő génekre, kromoszomális régiókra kell korlátozódnia. A szülők vizsgálata mind a CMA-, mind a WES-analízisek során kiemelt jelentőséggel bír, főleg azokban az esetekben, amelyeknél a kapott eltérés nem hozható egyértelmű összefüggésbe az ultrahang-eltérésekkel.
Következtetés: Fontos meghatározni azokat a paramétereket, amelyek alapján a magzati mintában talált kópiaszám-eltéréseket és WES-vizsgálattal igazolt variánsokat a leletben közöljük (figyelembe véve a nemzetközi ajánlásokat). Ezek alapján a praenatalis klinikai genetikai tanácsadáskor sokkal használhatóbb információk adhatók.
Orv Hetil. 2024; 165(14): 523–530.

Kulcsszavak: CMA (chromosomal microarray analysis), kromoszomális microarray-analízis, WES (whole-exome sequencing), teljesexom-szekvenálás, praenatalis diagnosztika

Summary of the first Hungarian experiences with prenatal chromosomal microarray analysis and whole-exome sequencing

Introduction: One of the major technological innovations of the last decade has been the proliferation of high-throughput molecular genetic testing methods, such as chromosomal microarray analysis (CMA) and whole-exome sequencing (WES) in prenatal diagnostics.
Objective: Over the past 5 years, our working group has performed more than 252 prenatal examinations, indicated by ultrasound abnormalities of varying severity. Depending on the results of classical cytogenetic studies, we performed high-resolution CMA and WES analyses, with the aim to map the proportion of excess genetic information in the Hungarian population, as described in the literature.
Method: CMA studies were performed using the "GeneChip System 3000 Instrument" platform with SNP-based comparative hybridization. We also performed next-generation sequencing of the whole human exome using Ion-Torrent and Illumina platforms.
Results: A total of 252 fetal CMA examinations were performed and 42% showed some loss or gain, of which 22% showed pathogenic abnormalities. We performed WES in 42 CMA-negative cases, of which 9 (21.4%) were identified as pathogenic abnormalities supporting the inheritance process, with presumed association with fetal phenotype, based on the ClinVar database or ACMG classification.
Discussion: Given the indirect nature of fetal phenotype assessment, prenatal CMA and WES analysis should be limited primarily to genes and chromosomal regions associated with ultrasound-identifiable symptoms. Parental examination is of paramount importance in both CMA and WES analyses, especially in cases where the resulting disorder cannot be clearly associated with ultrasound abnormalities.
Conclusion: It is important to define the parameters by which copy number variations are detected in fetal samples. Recommendations for reporting variants confirmed by WES testing should also be given (taking international recommendations into account). These will provide more useful information for prenatal clinical genetic counselling.

Keywords: CMA (chromosomal microarray analysis), WES (whole-exome sequencing), prenatal diagnostics

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Rövidítések

ACMG = (American College of Medical Genetics and Genomics) Amerikai Orvosi Genetikai és Genomikai Kollégium; ADGRV1 = (adhesion G protein-coupled receptor V1); ANO5 = (anoctamin 5); ATAD3A = (ATPase family AAA domain containing 3A); BP = (breakpoint) töréspont; BSCL2 = (BSCL2 lipid droplet biogenesis associated, seipin); CAPN3 = (calpain 3); CBL = Cbl protoonkogén; CC2D2A = (coiled-coil and C2 domain containing 2A); CMA = (chromosomal microarray analysis) kromoszomális microarray-analízis; CNV = (copy number variation) kópiaszám-eltérés; DNS = dezoxiribonukleinsav; EBP = (EBP cholestenol delta-isomerase) EBP koleszt enol delta-izomeráz; GJB2 = (gap junction protein beta 2); Kb = kilobázispár; KIF21A = (kinesin family member 21A); LCR = (low copy repeat) kis kópiaszámú ismétlődés; Mb = megabázispár; NAHR = (non-allelic homologous recombination) nem allélikus homológ rekombináció; NGS = (new-generation sequencing) újgenerációs szekvenálás; NHEJ = (non-homologous end joining) nem homológ végek összekapcsolása; NIPT = (non-invasive prenatal test) nem invazív praenatalis teszt; PTPN11 = (protein tyrosine phosphatase non-receptor type 11); PUS3 = (pseudouridine synthase 3);

SNP = (single-nucleotid polymorphism) egy nukleotidot érintő polimorfizmus; SOS1 = (SOS ras/rac guanine nucleotide exchange factor 1); TEK = (TEK receptor tyrosine kinase); TNNT3 = (troponin T3, fast skeletal type); TPM3 = (tropomyosin 3); VCF = velocardiofacialis; VMCM = mucocutan vénás malformációk; VUS = (variant of uncertain significance) ismeretlen jelentőségű eltérés; WES = (whole-exome sequencing) teljesexom-szekvenálás

A praenatalis diagnosztika célja a méhen belül fejlődő magzat rendellenességeinek feltérképezése, időben történő diagnosztizálása, a házaspárok számára azon lehetőség biztosítása, hogy a törvényi kereteken belül dönthessenek magzatuk betegsége esetén annak sorsáról. A praenatalis szűrés és diagnosztika jelenlegi két alappillére a nagy felbontású ultrahangtechnika és a célzott biopszia (lepényszövet, magzatvíz, esetleg magzati szövetek) során nyert minta hagyományos citogenetikai vagy molekuláris biológiai elemzése. A terhesség 10–14. hete között elvégezhető a chorionboholy-mintavétel, a 15–20. hete között pedig az amniocentesis. Évtizedek óta a lepényi, illetve a magzatvízmintából elvégzett hagyományos citogenetikai vizsgálat (kariotipizálás) a praenatalis genetikai diagnosztika „arany standard" módszere, amely a magzati kromoszómák számbeli és durva szerkezeti rendellenességeinek kimutatására alkalmas. A módszer által a magzati testi és nemi kromoszómák számbeli (például triszómiák és monoszómiák) és bizonyos szerkezeti rendellenességeik (például törések, transzlokációk, többletek, hiányok) kimutatása lehetséges. A jelenleg alkalmazott, ún. hagyományos kromoszómavizsgálat felbontási határa kb. 10–12 Mb nagyságú. Az ennél kisebb méretű eltérések nem mutathatók ki fénymikroszkóp-alapú klasszikus citogenetikai módszerekkel [1].

A hagyományos citogenetikai vizsgálattal nem felismerhető, 1–5 Mb tartományú, több gént is érintő eltérések következményeképpen gyakran sokrétű, súlyos tünetegyüttesek alakulhatnak ki, melyeket microdeletiós/mikroduplikációs kórképeknek nevezünk. Ezek a szindrómák általában súlyos kórállapotok, amelyek értelmi fogyatékossággal, idegrendszeri tünetekkel, komplex szervi rendellenességekkel járhatnak. A kromoszomális microarray-analízis (CMA) módot biztosít a kis méretű hiányok/többletek (microdeletiók és mikroduplikációk, illetve kópiaszám-eltérések [CNV-k]) azonosítására [2, 3].

Az újgenerációs szekvenálás (NGS) a 2010-es évektől óriási előrelépést biztosított a néhány nukleotidot érintő eltérések azonosítására és azok megismerésére a különböző betegségekkel összefüggésben a teljesexom-szekvenálás (WES) segítségével. Ez a nagy áteresztőképességű újgenerációs módszer a költségek csökkenésével és a bioinformatikai algoritmusok fejlődésével mára a praenatalis diagnosztika megkerülhetetlen részévé vált [4]. A WES-vizsgálat pozitív hozzáadott értéke a CMA és a kariotipizálás negatív eredménye után 8–10% körüli [5, 6], de egyes közlemények szerint akár a 80%-ot is elérheti, és erősen korrelál a magzati rendellenességek számával [7, 8].

Célkitűzés

Munkacsoportunk a saját tapasztalatai alapján a nemzetközi előírásoknak megfelelően kialakított egy diagnosztikai algoritmust, amely szerint praenatalis genetikai vizsgálatok indikációjakor i) elsőként hagyományos kariotipizálást indítunk, ennek negatív eredménye esetén, ii) nagyobb felbontóképességű CMA következik, amelynek negativitása esetén iii) WES-vizsgálattal folytatva a diagnosztikai sort, az egypontos nukleotideltérések azonosíthatók. Célunk a fentiek szerint végzett eddigi vizsgálatainkból leszűrhető következtetések elemzése.

Módszer

A CMA-vizsgálatokat GeneChip System 3000 Instrument (Affymetrix, Santa Clara, CA, USA) platformmal végeztük: a hibridizációhoz a GeneChip Hybridization Oven 645 (Thermo Fisher Scientific, Waltham, MA, USA) hibridizációs kamrát, a mosási lépéshez a GeneChip Fluidics Station 450 (Thermo Fisher Scientific) készüléket, a jelintenzitás mérésére a GeneChip Scanner 3000 7G (Thermo Fisher Scientific) készüléket használtuk.

A vizsgálat egy SNP-alapú komparatív genomiális hibridizációs módszeren alapul. A DNS-mintát NspI enzimmel különböző méretű darabokra emésztjük, majd ezekhez egy adapter molekulát kötünk, amellyel teljesgenom-amplifikálást végzünk. Az amplifikált mintát jelöljük (biotinnal), majd hibridizáljuk az array szilárd fázisán lévő 25 nukleotidból álló oligonukleotid-próbákhoz, amelyekkel a teljes humán genomot lefedjük. Ezután a mosási lépésnél a be nem kötődő DNS-darabokat és egyéb szennyeződéseket eltávolítjuk a szilárd fázisról, és a biotinnal jelölt és a próbákhoz bekötődött DNS-darabokat sztreptavidin-fikoeritrin komplexszel festjük. Így létrejön egy biotin-sztreptavidin-fikoeritrin komplex, amely gerjeszthető, és az 572 nm-es tartományban emittál. A jelintenzitás mérésekor az emittálójelet a kópiaszám arányának megfelelően detektálja a készülék, és a végső analízissel vizualizálhatjuk az egyes kromoszómákat, amelyekben az esetleges hiányt vagy többletet is megjelöli a rendszer.

Az általunk elvégzett NGS során a teljes humán exom szekvenciáját meghatároztuk 42 praenatalis minta esetében. A szekvenálás IonTorrent (Thermo Fisher Scientific) és Illumina (Illumina, San Diego, MA, USA) platformokon történt. A megfelelő minőségű és mennyiségű genomiális DNS kivonását követően, a könyvtárkészítés első lépéseként felsokszoroztuk a teljes humán exomot, majd több tisztítási és jelölési lépés után, amelyek lehetővé tették a multiplex párhuzamos szekvenálást, a kész könyvtárak koncentrációját ellenőriztük. Ezután a könyv-

tárakat ekvimoláris mennyiségben felvittük a szekvenálófelületre (IonChip vagy FlowCell). A szekvenálást követően a keletkezett szekvenciák illesztését a referenciagenomhoz (hg37) és azt követően a variánshívást GATK v4.1.4.1 szoftverrel végeztük, a talált variánsok annotálása és interpretálása során a 'Franklin by Genoox'-ot használtuk.

A genetikai vizsgálat elvégzéséhez a páciensek minden esetben beleegyező nyilatkozatot írtak alá, és az adatok retrospektív feldolgozása anonimizált módon történt.

Eredmények

Összesen 252 praenatalis citogenetikai vizsgálatot végeztünk. A CMA-vizsgálat indikációja az esetek 92,8%-ában valamilyen strukturális magzati ultrahangeltérés volt *(1. táblázat)*. A WES-vizsgálat javallata általában a negatív karyotypus és CMA, illetve a genetikai okot valószínűsítő ultrahangeltérés volt. Az ultrahangeltérések közül a leggyakoribbak a magzati hydrops, a cysticus hygroma, valamint a vastag nyaki redő (40,60%), a craniospinalis és craniofacialis rendellenességek (20,51%), valamint a cardiovascularis rendellenességek (17,95%) voltak *(2. táblázat)* [9–11].

A vizsgált magzati minták közül 147 (58%) esetben nem mutattunk ki semmilyen eltérést a CMA-módszerrel, az Affymetrics Optima közepes felbontóképességű array alkalmazásával. A többi esetben 105-nél (42%) mutattunk ki eltérést (hiányt vagy többletet) a CMA-vizsgálattal, amelyek közül 22%-ban igazoltunk kóros eltérést, 6 esetben (2%) pedig teljes kromoszómát érintő triszómiát a 21. kromoszómán (5 magzatnál), illetve a 18. kromoszómán (1 magzatnál). A leggyakoribb eltéréseket és azok elhelyezkedését a *3. táblázat* tartalmazza.

Három esetben a szülők kiegyensúlyozott transzlokációt hordoztak, amely a magzatnál kiegyensúlyozatlan állapotot idézett elő: i) az apánál a t(9;20)(p24;p12) és a magzatnál a 9p24.3p24.1 régióban microdeletio, a 20p13p12.1 régióban 14,755 Mb méretű mikroduplikáció; ii) az anyánál a t(4;5)(p16;p15) és a magzatnál a 4p16.3p16.2 régióban 4,984 Mb méretű microdeletio és az 5p15.33p14.3 régióban 19,389 Mb méretű mikroduplikáció; iii) az anyánál a t(4;8)(p16;p23) és a magzatnál a 4p16.3p15.2 régióban 23,697 Mb méretű mikroduplikáció és a 8p23-3p23.1 régióban 6,474 Mb méretű microdeletio.

Egy esetben igazoltunk „összetett heterozigótaságot", amelyet a CMA-val az anyai allélon 4,138 Mb méretű (arr[GRCh38]12q12(38620776_42758565)x1) heterozigóta deletióként azonosítottunk, és az apai allélon WES-analízissel az ebben a régióban elhelyezkedő *KIF21A* génben 1 bázist érintő deletio heterozigótahordozóságát mutattuk ki [12]. A két kóros eltérés együttes hatásaként a családban két érintett magzatnál súlyos, már magzati korban kimutatható kóros tüneteket

1. táblázat | CMA- és WES-vizsgálataink indikációi (252 eset)

	Eset (n)	%
Magzati strukturális ultrahangeltérés (lásd *2. táblázat*)	234	92,8%
Pozitív NIPT-lelet	2	0,8%
Terhelő anamnézis	4	1,6%
Kariotipizálással igazolt CNV esetén (töréspontok meghatározása)	2	0,8%
Kariotipizálással igazolt marker kromoszóma	2	0,8%
Szülőknél kiegyensúlyozott transzlokáció	3	1,2%
Spontán vetélés (abortumvizsgálata)	3	1,2%
Halvaszülés (24. hét utáni elhalás)	2	0,8%
Összesen	252	100%

CMA = kromoszomális microarray-analízis; CNV = kópiaszám-eltérés; NIPT = nem invazív praenatalis tesztelés; WES = teljesexom-szekvenálás

2. táblázat | CMA- és WES-vizsgálatok strukturális magzati ultrahangeltérés miatt

Szervrendszer	Eset (n)	%
Magzati hydrops, cysticus hygroma, vastag tarkóredő	95	40,60%
Craniospinalis és craniofacialis rendellenességek	48	20,51%
Cardiovascularis rendellenességek	42	17,95%
Egyéb thoracalis rendellenességek	6	2,56%
Hasfali és hasi rendellenességek	7	2,99%
Urogenitalis rendellenességek	8	3,42%
Végtag-anomáliák és csontosodási zavarok	14	5,98%
Egyéb strukturális ultrahangeltérések	14	5,98%
Összesen	234	100%

CMA = kromoszomális microarray-analízis; WES = teljesexom-szekvenálás

3. táblázat | A leggyakoribb kópiaszám-eltérések

Kópiaszám -eltérés	Jellemzés	Eset (n)	%
16p11.2	Ismert LCR/SD régió/VUS	23	30,26%
3q24q29:	Ismert LCR/SD régió*	8	10,52%
8p22p24.3:	LOH polimorf régió**	8	10,52%
16p13.11p12.3:	Ismert LCR/SD régió	7	9,21%
Xq21.31q21:	VUS	7	9,21%
15q11.2q13.3:	Ismert BP- (1–3) régió*	6	7,89%
4p16.3p16.2:	Telomer régió	5	6,58%
9p24.3p24.3:	Telomer régió	4	5,27%
22q11.21:	DiGeorge-régió	4	5,27%
20q13.33q14.33	Telomer régió	4	5,27%
Összesen		76	100%

*LCR = alacsony kópiaszámú ismétlődés
**LOH = heterozigótaság elvesztése

BP = töréspont; LCR = alacsony kópiaszámú ismétlődés; SD = szegmentális duplikáció; VUS = ismeretlen jelentőségű eltérés

írtak le (asphyxiás thorax szindróma, orrgyöki oedema, a magzat törzsén is megfigyelhető oedema, borderline cerebralis ventriculomegalia). 3 esetben elhalt magzati szövetből izolált DNS-mintából végeztük a CMA-t, és 1 esetben triszómiát igazoltunk a 18. kromoszómán, 1 esetben pedig részleges triszómiát a 9. kromoszómán *(1. táblázat)*.

A munkacsoportunk által alkalmazott vizsgálati sorrendben, azokban az esetekben, amelyeknél a klasszikus citogenetika és a CMA-k sem mutattak ki eltérést, további vizsgálatként a WES-analízissel igyekeztünk feltérképezni az ismert kóroki pontmutációt. Az általunk elvégzett NGS során a teljes humán exom szekvenciáját meghatároztuk 42 praenatalis minta esetében. A WES-vizsgálattal 9 esetben (21,4%) azonosítottunk az öröklésmenetet támogató, a magzati fenotípussal feltételezhető összefüggésben lévő kóros eltérést a ClinVar adatbázis vagy az ACMG-klasszifikáció alapján.

A pozitív eredmények közül 2 esetben a *PTPN11* génben (c.922A>G p.Asn308Asp és c.923A>C p.Asn308Thr), 1 esetben az *SOS1* génben (c.1644T>A p.Ser548Arg) és 1 esetben az *EBP* génben (c.338+1_338+2del) azonosítottunk kóros *de novo* variánst. További 1 esetben az érintett édesanya ugyanazt az arthrogryposissal társult eltérést hordozta, mint a tünetes magzat (TNNT3:c.188G>A p.Arg63His). 2 esetben az autoszomális recesszív öröklésmenetet támogató összetett heterozigóta statust láttunk súlyos ciliopathiával (CC2D2A:c.4552C>T p.Arg1518Trp és c.4675-1G>C) és multiplex arthrogryposissal társuló súlyos fetalis akinesiát. Mindkét esetben a családi szegregációs vizsgálat megerősítette az öröklésmenetet. 2 további esetben az azonosított variánsokat (CBL c.1754G>T p.Arg585Leu és TEK c.2744G>A p.Arg915His) olyan génekben írták le, amelyeknél a génekkel összefüggésbe hozott fenotípus megfeleltethető ugyan az ultrahangképnek, de a variáns kóroki szerepét a rendelkezésre álló adatok nem támasztják alá egyértelműen.

A WES-vizsgálatok során 18 esetben azonosítottunk olyan véletlen/másodlagos találatot, amely a ClinVar adatbázis vagy az ACMG-klasszifikáció szerint kóros vagy valószínűleg kóros besorolású *(4. táblázat)*. Ezekben az esetekben a génekkel összefüggésbe hozott fenotípusos jegyek nem feleltek meg egyértelműen az ultrahangképnek, de mindegyik súlyos kórképpel társult.

4. táblázat Feltételezett incidentális találatok a praenatalis WES-vizsgálati csoportban

Gén	Variáns	ClinVar szerinti besorolás	ACMG-klasszifikáció
ATAD3A	c.229C>G p.Leu77Val	P/VUS	LP
ANO5	c.2272C>T p.Arg758Cys	P/LP	LP
CFI	c.772G>A p.Ala258Thr	P/LP	LP
ROBO4	c.190C>T p.Arg64Cys	P	P
PROS1	c.701A>G p.Tyr234Cys	P/LP	LP
ANO5	c.692G>T p.Gly231Val	P/LP	LP
GATA4	c.487C>T p.Pro163Ser	P/VUS	LP
GATA4	c.34G>C p.Gly12Arg	VUS	LP
RPS7	c.298A>T p.Ile100Phe	–	LP
PAH	c.506G>A p.Arg169His	LP	P
F2	c.*97G>A	P/VUS	LP
ADGRV1	c.9623+1G>A	LP	P
F11	c.1693G>A p.Glu565Lys	LP/VUS	LP
BSCL2	c.974dupG p.Ile326fs	P/LP	P
PUS3	c.212A>G p.Tyr71Cys	P/VUS	LP
GJB2	c.35del p.Gly12ValfsTer2	P	P
CAPN3	c.550del p.Thr184ArgfsTer36	P	P
TPM3	c.253G>T p.Glu85Ter	–	LP

ACMG = az Amerikai Orvosi Genetikai és Genomikai Kollégium által megalkotott variánsosztályozási rendszer alapján a Franklin-platform által számított variánsklasszifikáció; ClinVar = a ClinVar adatbázis alapján a jelenlegi tudásunk szerinti legvalószínűbb besorolás; LP = valószínűleg kóros (patogén); P = kóros (patogén); VUS = ismeretlen jelentőségű eltérés; WES = teljesexom-szekvenálás

Megbeszélés

A CMA-vizsgálatok során 99 esetben igazoltunk microdeletiót vagy mikroduplikációt a magzati mintában. Az esetek nagy száma azt is igazolta, hogy a szakirodalomban leírt genomiális szerkezeti formák befolyásolhatják a microdeletiók és mikroduplikációk előfordulási gyakoriságát. Ilyen szerkezeti elemek például az ún. „low copy repeat" (LCR)/szegmentált duplikációs régiók: ezek olyan, 10–300 Kb méretű ismétlődő szekvenciák, amelyek meioticus osztódáskor az ún. nem allélikus homológ rekombináció (NAHR) során az utódsejtben microdeletiókat vagy mikroduplikációkat alakíthatnak ki [13]. Így például a szakirodalomban leírtak alapján a 16. kromoszóma p13.1, p12.3, p12.2, p11, q22.2 és q23 régióiban olyan LCR-ek találhatók, amelyek változatos méretű és különböző töréspontú átrendezéseket idézhetnek elő a meioticus osztódáskor. Bár ezek az LCR-ek a 16. kromoszóma pericentromer régiójának ún. génszegény környezetében helyezkednek el, az érintett génektől függően bizonyos esetekben mégis kóros fenotípushoz vezethetnek. Az általunk vizsgált magzati mintákban a legnagyobb gyakorisággal mutattuk ki az ebben a régióban található 16p11.2 microdeletiót vagy mikroduplikációt (23%). A szakirodalom alapján ez az eltérés nagy valószínűséggel nem jár tünetekkel (VUS-kategória) [14].

A szegmentális duplikációk (legismertebb formájuk az ún. tandem duplikáció) a genom több mint 6%-át teszik ki. Ezek az ismétlődések szintén okozhatnak a genomban strukturális változásokat, mind a NAHR, mind a nem homológ végek összekapcsolása (NHEJ) révén. Ez utóbbi következményeként, a kettőzött DNS-darabka – extrakromoszomális helyzetbe kerülve, majd visszail-

leszkedve a genomba – kialakíthat DNS-többletet vagy -hiányt, illetve az inszertálódás során, ha az 1 gént érint, monogénes kóroki fenotípust is [15].

Vizsgált magzati mintáinkban a negyedik leggyakoribb (4%-os) előfordulással találtunk többletet vagy hiányt a jól ismert, kóros fenotípussal járó 22q11.21 régióban, amelynél szintén a fent említett LCR szegmentált duplikációs régiók jelenléte áll a microdeletiók vagy mikroduplikációk kialakulásának hátterében. A vizsgált minták közül mind deletiót, mind duplikációt igazoltunk: az igazolt mikroduplikáció az LCR22-2 (A) és az LCR22-3b (C) régiókat érintette. A postnatalis esetekben a kóros fenotípust fejlődési és intellektuális elmaradás, fizikális eltérések, mint például hypotonia, közepesen dysmorphiás arc, valamint microcephalia jellemezte.

Az igazolt deletiós magzati esetek a 22q11.21 deletiós szindrómának (DiGeorge/VCF) feleltek meg. Ezekben a proximális és centrális régiók érintettsége volt kimutatható (LCR22-2 [A] – LCR22-5 [E]) [16, 17]. A 15. kromoszóma 15q11.2-q13.1, ún. kritikus régiója, a BP1–BP3 és a BP2–BP3 bármilyen CNV-je (microdeletio és mikroduplikáció) kockázatot jelenthet a fejlődéselmaradással járó kórképekre, illetve az autizmusspektrum-betegségre [18, 19]. A vizsgált magzati minták 6%-ában igazoltunk valamilyen eltérést ebben a régióban, amelyek esetleges neuropszichiátriai kórképekkel társulhatnak.

A szülőknél klasszikus citogenetikai módszerrel (G-sávozás) kimutatott kiegyensúlyozott transzlokáció esetén, a magzati ultrahangeltérés mellett, minden esetben invazív mintavétel szükséges, mivel a szülői kiegyensúlyozott transzlokáció könnyen kiegyensúlyozatlanná válhat a magzatban. A vizsgált populációban 3 esetben a szülők kiegyensúlyozott transzlokációt hordoztak, és a magzati mintákban ez mindhárom esetben kiegyensúlyozatlan állapotot idézett elő, amely kóros fenotípussal társult.

Kiemelendők azok az esetek, amelyeknél az ún. „összetett heterozigótaság” az ok a kóroki fenotípus kialakításáért. 1 esetben igazoltunk összetett heterozigótaságot CMA- és WES-vizsgálatokkal, amely súlyos kóros magzati ultrahangképpel is járt: az érintett magzati mintában az édesanyától örökölt heterozigóta 4,138 Mb méretű deletiót és az édesapától ebben a régióban található, egy bázist érintő deletiót találtunk, amely mint összetett heterozigóta állapot súlyos fenotípussal társult már magzati korban. Az összetett heterozigótaság hátterében véletlen események állhatnak, ezért is fontos a pontmutációk esetleges meglétének kizárása szekvenálással azokban az esetekben, amelyekben a CMA-vizsgálat heterozigótahiányt vagy -többletet igazolt, és autoszomális recesszíven öröklődő gén is érintett.

Azokban az esetekben, amelyeknél sem a klasszikus citogenetika, sem a CMA-vizsgálat nem igazolt kóroki eltérést, a következő lépésként WES-vizsgálatot végeztünk [20, 21]. Vizsgálataink során 9 esetben azonosítottunk a magzati fenotípusnak megfeleltethető génben feltételezhetően kóroki eltérést, 3 esetben Noonan-szindrómával és 1 esetben Noonan-szerű szindrómával összefüggésbe hozható génben *(PTPN11, SOS1, CBL)* aminosavcserével járó variánst. Az elvégzett családi szegregációs vizsgálatok során az utóbbi variánst a szülők is hordozták, így ennek besorolását módosítottuk.

Vázrendszeri rendellenességekkel összefüggésben 3 génben *(TNNT3, KIF21A, ABP)* találtunk feltételezhetően kóroki variánst. Az egyik esetben az autoszomális dominánsan öröklődő fenotípust megerősítette, hogy a tünetes édesanya is hordozta az eltérést, míg a másik, dominánsan öröklődő fenotípus esetében a variáns *de novo* eredete volt valószínűsíthető. A harmadik esetben a gyermek a hordozó szülőktől örökölte összetett heterozigóta formában az azonosított variánsokat. A kardiológiai tünetekkel jellemzett magzat esetében az azonosított variáns kóroki szerepe nem nyert megerősítést. Az irodalmi adatok alapján feltételezhető az ok-okozati viszony, a *TEK* génben általunk kimutatott variánst korábban egy VMCM-vel (vénás malformációk, többszörös bőr- és nyálkahártya-rendellenességgel) érintett családban azonosították, ahol az édesanya is érintett volt. Esetünkben a család visszautasította a további vizsgálatot.

1 esetben a magzati ultrahangvizsgálat alapján ciliopathia gyanújával összetett heterozigóta formában azonosítottunk két, feltételezhetően kóroki eltérést a *CC2D2A* génben, ahol a variánsok *transz*-helyzetét a családi szegregációs vizsgálat megerősítette. Az eredmény közlésekor magzati vizsgálat esetében is elsősorban a meglévő fenotípusos jegyek mentén zajlik a kiértékelés. Mivel ezek az információk praenatalisan csak közvetve vagy korlátozottan állnak rendelkezésünkre, és a terhesség során változhatnak, a variánslista további szűkítése célszerű. Erre alkalmas lehet az érintett génekhez társult betegségek öröklésmenete, illetve a betegségek megnyilvánulásának jellemző időpontja vagy a megjelenő fenotípus súlyossága [22, 23].

A bizonytalan jelentőségű variánsok (VUS), a véletlen és másodlagos találatok szintén komoly kihívást jelentenek a laboratóriumok és a klinikusok számára. A véletlen (incidentális) találatok a klinikai indikációtól függetlenül azonosított variánsok. A másodlagos találatok az ACMG szakértői által összeállított és validált génlistán szereplő eltérések, amelyek nem kapcsolódnak az elsődleges fenotípusos megjelenéshez, de az orvosi döntéshozatal folyamatában felhasználható információt hordozhatnak. Az általunk vizsgáltak között 18 esetben azonosítottunk autoszomális domináns kórképpel összefüggésben a ClinVar adatbázis vagy az ACMG-klasszifikáció szerinti kóros vagy valószínűleg kóros eltérést [24, 25]. Az azonosított variánsoknál megvizsgáltuk a fenotípusos jegyeket, az irodalmi adatokat és – ahol volt lehetőségünk – a szülői mintákat is [26]. Az áttekintés során az azonosított variánsokat nagyobb csoportokba tudtuk sorolni. A legtöbb, általunk azonosított variáns patogenitására vonatkozó adat autoszomális recesszív kórkép hátterében került közlésre, illetve néhány esetben az autoszomális domináns szerep még nem kellőképpen volt alátá-

masztva (például *BSCL2, PUS3, GJB2, CAPN3, TPM3, ADGRV1, ANO5, ATAD3A* gének). Ezeknél a géneknél a klinikai újraértékelés, a magzati fenotípus ismételt elemzése, a különböző molekuláris technikák összehasonlítása, a családi szegregációs vizsgálat és az irodalmi adatok újbóli áttekintése segítheti a döntést az eredmény közlésére vonatkozóan [27–29].

Következtetés

A praenatalis vizsgálatokban a klasszikus citogenetikai vizsgálatok mellett egyre inkább teret hódítanak a nagy felbontóképességű eljárások, mint a CMA- és WES-vizsgálati módszerek. A nemzetközi protokollokat alapul véve, saját tapasztalataink alapján, terhelő anamnézis esetén a CMA-vizsgálat hasznosnak és gyorsan kivitelezhetőnek bizonyult a pozitív ultrahangvizsgálat és/vagy NIPT-eredmény megerősítésére. A CMA-módszer alkalmas továbbá klasszikus citogenetikai vizsgálattal igazolt, de sávozási technikával nem vagy nehezen meghatározható eredetű többlet eredetének pontos meghatározására is, és megbízhatóan alkalmazható szám feletti marker kromoszómák eredetének meghatározására és szülőknél kimutatott kiegyensúlyozott transzlokáció esetén a magzatban esetleg kialakuló kiegyensúlyozatlan eltérések felismerésére is.

Vetélések esetén azokban az esetekben alkalmazhatjuk a CMA-módszert, amelyeknél a klasszikus citogenetikai módszerekkel nem lehet vizsgálni a sejteket, mivel esetleg a sejtek már nekrotizált állapotba kerültek, és nem képesek osztódni. Tapasztalataink alapján az ultrahangvizsgálattal megállapított magzati elhalást követően rövid időn belül kell az abortumból a DNS-izolálást elvégezni, mert az esetleges nekrotikus folyamatok beindulása befolyásolhatja a CMA-vizsgálat eredményességét.

Sok esetben mutattunk ki microdeletiót vagy mikroduplikációt. A nemzetközi ajánlásokat figyelembe véve közöltük ezeket a leletekben: i) a kóros és nagy valószínűséggel kóros eltéréseket minden esetben közöltük, ii) méret alapján mikroduplikáció esetén az 1 Mb méretnél nagyobb eltérést, illetve microdeletio esetén a 0,5 Mb méretnél nagyobb eltérést ismertettük, iii) az ACMG-standard szerint a CNV-re számolt pontérték alapján is meghatározható az adott eltérés patogenitása, iv) adatbázisokat is felhasználtunk (Decipher, ClinVar, ClinGen), hogy az érintett régióban leírt postnatalis esetek, valamint az érintett génekre jellemző tulajdonságok alapján (például dózisszenzitív az érintett gén, vagy nem) fenotípus-előrejelzést adhassunk.

A WES-vizsgálatok indikációja jelenleg még nem olyan széles körű, mint a CMA-vizsgálatoké, de minden olyan esetben, amelynél a genetikai eredet valószínűsíthető, és az előzetes hagyományos kariotipizálás, valamint CMA-vizsgálat negatív eredménnyel zárult, javasolt a WES elvégzése. Ahol a terhesség előrehaladott kora szükségessé teszi, javasolható a CMA- és a WES-vizsgálatok egyidejű indítása. Abban az esetben, ha a családi kórtörténet vagy a magzati fenotípus nukleotidszintű genetikai hátteret feltételez, a WES megelőzheti a CMA-t [6, 30].

Összességében, a CMA 22%-ban (252 esetből), a WES pedig 21,9%-ban (42 esetből) adott többletinformációt a klasszikus citogenetikához képest. A nemzetközi szakirodalomban megjelent publikációkban erre eltérő százalékokat adnak meg: CMA-ra 6–10%-os és 20%-os értéket [31, 32], míg WES-re a legalacsonyabb 6,2% és a legmagasabb 57,1% [33]. A különbségek a különböző platformok alkalmazásából adódhatnak: CMA esetén a nagyobb felbontóképességű array több eltérést képes kimutatni, valamint a leletben közölt eltérések méretének megválasztásával is eltérő százalékos adatok kaphatók. A G-sávozás, a CMA és a WES összességében kb. 35%-ban tud genetikai érintettséget azonosítani, míg a fennmaradó 65%-ban teratogén hatások állhattak a kóros ultrahangeltérés és a magzati fejlődési rendellenesség hátterében. Ezek alapján munkacsoportunk fontosnak tartotta, hogy – a nemzetközi ajánlásokat figyelembe véve és a nemzeti sajátságokat hozzátéve – egységes praenatalis diagnosztikai protokollt alakítson ki a CMA- és WES-vizsgálatokra vonatkozólag.

Fontos megemlíteni, hogy sem a CMA, sem az egész genomra kiterjedő szekvenálási módszerek nem képesek minden genetikai rendellenességet kimutatni vagy teljesen kizárni. A ritka rendellenesség fennmaradó kockázata, különösen a többszörös veleszületett rendellenességek esetén, fontos szempont a tanácsadás során.

Megjegyzés: A vizsgálatok a napi rutin részét képezik, amelyhez nem szükséges kutatási engedély. A genetikai vizsgálatokhoz minden esetben beleegyező nyilatkozatot töltöttek ki a betegek, és ezt az adott intézeti egység a törvényben meghatározott időtartamig tárolja.

Anyagi támogatás: A közlemény megírásához a szerzők anyagi támogatásban nem részesültek.

Szerzői munkamegosztás: P. H.: CMA-vizsgálatok kivitelezése és kiértékelése, az eredmények összegzése. I. A.: WES-vizsgálatok kiértékelése és összegzése. K. J., T. B., B. B.: A tudományos eredmények összegzése és értékelése. N. S., B. A., H. E., T. O., T. Zs.: Mintavétel, genetikai tanácsadás, az eredmények összegzése. Á. K.: WES-vizsgálatok kivitelezése, kiértékelése és az eredmények összegzése. E. T., L. V., K. V., K. Zs., T. E.: Genetikai tanácsadás, az eredmények összegzése. F. M., M. P.: Mintavétel, genetikai tanácsadás. L. P.: A tudományos eredmények kiértékelése és összegzése, valamint a nemzeti stratégiák meghatározása. T. I.: A tudományos eredmények kiértékelése és összegzése, valamint a nemzeti stratégiák meghatározása. A cikk végleges változatát valamennyi szerző elolvasta és jóváhagyta.

Érdekeltségek: A szerzőknek a cikkel kapcsolatosan nincsenek érdekeltségeik.

Köszönetnyilvánítás

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Irodalom

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