

ORIGINAL ARTICLE

A two-year prospective study assessing the performance of fetal chromosomal microarray analysis and next-generation sequencing in high-risk pregnancies

Konstantin Ridnõi^{1,2}  | Kai Muru^{2,3} | Maria Keernik³ | Sander Pajusalu^{2,3,4}  |
Eva-Liina Ustav⁵ | Pille Tammur³ | Triin Mõlter-Väär³ | Tiina Kahre^{2,3} |
Ustina Šamarina³ | Karin Asser⁶ | Ferenc Szirko¹ |
Tiia Reimand^{2,3,7} | Katrin Õunap^{2,3} 

¹Centre for Perinatal Care, Women's Clinic, East-Tallinn Central Hospital, Tallinn, Estonia

²Department of Clinical Genetics, Institute of Clinical Medicine, University of Tartu, Tartu, Estonia

³Department of Clinical Genetics, United Laboratories, Tartu University Hospital, Tartu, Estonia

⁴Department of Genetics, Yale University School of Medicine, New Haven, CT, USA

⁵Women's Clinic, Tartu University Hospital, Tartu, Estonia

⁶Radiology Clinic, Tartu University Hospital, Tartu, Estonia

⁷Department of Biomedicine, Institute of Biomedicine and Translational Medicine, University of Tartu, Tartu, Estonia

Correspondence

Konstantin Ridnõi, Centre for Perinatal Care, Women's Clinic, East-Tallinn Central Hospital, 18 Ravi Street, Tallinn 10138, Estonia.

Email: konstantin.ridnoi@itk.ee

Abstract

Background: Introduction of cell-free fetal DNA (cff-DNA) testing in maternal blood opened possibilities to improve the performance of combined first-trimester screening (cFTS) in terms of better detection of trisomies and lowering invasive testing rate. The use of new molecular methods, such as chromosomal microarray analysis (CMA) and next-generation sequencing (NGS), has shown benefits in prenatal diagnosis of chromosomal and genetic diseases, which are not detectable with cff-DNA screening, but require an invasive procedure.

Methods: The objective of this study was to evaluate prospectively during two years performance of CMA and NGS in high-risk pregnancies. Initially, we investigated 14,566 singleton pregnancies with cFTS. A total of 334 high-risk pregnancies were selected for CMA diagnostic performance evaluation and 28 cases of highly dysmorphic fetuses for NGS analysis. CMA study group was divided into two groups based on the indications for testing; group A patients with high-risk for trisomies after cFTS, but normal ultrasound and group B patients who met criteria for CMA as a first-tier diagnostic test.

Results: The diagnostic yield of CMA was overall 3.6% (1.6% in Group A and 6.0% in Group B). In NGS analysis group, we report diagnostic yield of 17.9%.

Conclusion: The use of CMA in high-risk pregnancies is justified and provides relevant clinical information in 3.6% of the cases. NGS analysis in fetuses with multiple anomalies shows promising results, but more investigations are needed for a better understanding of practical applications of this molecular diagnosis method in prenatal settings.

Tiia Reimand and Katrin Õunap are supervisors with equal input for the study.

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KEYWORDS

chromosomal microarray, fetal evaluation, next-generation sequencing, prenatal diagnosis, ultrasound anomalies

1 | INTRODUCTION

Over the past 30 years, prenatal screening for chromosomal diseases has undergone enormous development; for example, measuring maternal serum markers during the second trimester, combining different first-trimester serum markers with ultrasound examination, and sequencing cell-free fetal DNA in maternal plasma.

In addition to chromosomal investigations, molecular diagnostics for monogenic disorders has evolved in parallel facilitating even more precise diagnoses in prenatal diagnostics. Initially, a chromosomal microarray analysis (CMA) (Wapner et al., 2012) was implemented, which is a first-tier prenatal diagnostic test already in some countries (Muys et al., 2018; Vogel et al., 2018). Approximately 2–3% of all pregnancies are complicated by fetal structural anomalies (Edwards & Hui, 2018). Modern technologies, such as next-generation sequencing (NGS) have also shown promising results, with a high diagnostic yield in selected prenatal cases (Deden et al., 2020; Ferretti et al., 2019).

We conducted a prospective two-year study to evaluate the performance of molecular methods in prenatal diagnostics in Estonia during the years 2017–2018. The aim of our study was to assess the diagnostic effectiveness of CMA and NGS panel testing in high-risk pregnancies after cFTS and ultrasound examinations, performed according to the Estonian national guideline (Ustav et al., 2016).

2 | MATERIALS AND METHODS

2.1 | Study groups

CMA analysis study group

During the study period, cFTS was performed in 14,566 singleton pregnancies. We recruited 334 women for the CMA diagnostic effectiveness evaluation (Figure 1). These women were further divided into two subgroups. Group A (184 women) included all patients at high-risk of $\geq 1:100$ for trisomies after cFTS, but with nuchal translucency (NT) below 3.5 mm, no ultrasound malformations and normal conventional karyotype. All women in Group A were counselled before the invasive procedure and an additional written informed consent was taken before performing CMA on fetal DNA. Group B (150 women) included all patients who met the criteria for CMA as a first-tier diagnostic test. CMA in Estonia is performed as a first-tier diagnostic test after an invasive procedure, if one of the following clinical indications is met: NT ≥ 3.5 mm; fetal malformations seen on ultrasound; by the decision of a medical geneticist; or a family history of balanced translocation in one parent (Ustav et al., 2016). All women in Group B gave written consent for a regular medical service.

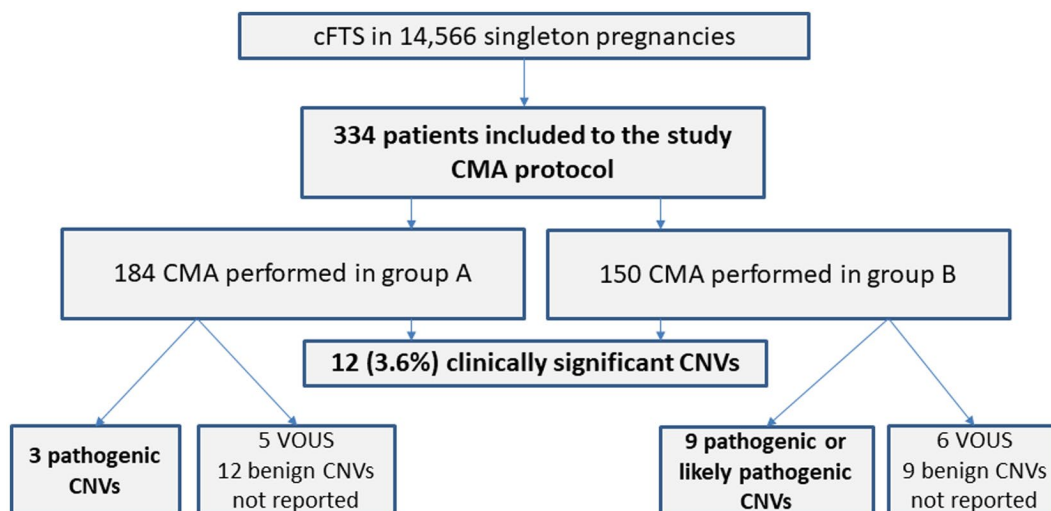


FIGURE 1 CMA study group. cFTS- combined first trimester screening, CMA-chromosomal microarray analysis, CNV-copy-number variant, VOUS- variant of uncertain clinical significance

NGS analysis study group

During the study period, a total of 28 cases were selected for the NGS panel analysis group. Inclusion criteria were: fetal brain anomalies, non-immune fetal hydrops, combined heart defects, or multiple fetal anomalies suggestive of an underlying genetic syndrome (Table 1). The decision to perform NGS panel analysis was made by a clinical geneticist who also counselled the patient, and an additional written informed consent for performing NGS analysis on fetal DNA was taken. CMA was performed in every case prior to the NGS analysis. Only two pregnancies resulted in live births; all others were terminated upon patients' request and medical indication before the 22nd week of pregnancy, according to Estonian law.

2.2 | Methods

Ethical compliance

The present study was approved by the Research Ethics Committee of the University of Tartu (protocol 263/M-19 17.10.2016).

CMA methods

Genomic DNA was extracted directly from AC or CVS or from a cultured sample. CMA was performed using Illumina HumanCytoSNP-12 BeadChips (Illumina Inc.). Genotype analysis was carried out using GenomeStudio software v2011.1 (Illumina Inc.) with additional input from QuantiSNP v2.3 software.

Copy number variants (CNV) were classified as follows: pathogenic, likely pathogenic, variant of uncertain clinical significance (VOUS) and benign or likely benign (Nowakowska, 2017). In prenatal cases, benign and likely benign findings or long contiguous stretches of homozygosity (LCSH) of any size were not reported.

Several online databases were used in the decision-making: OMIM, human genome browsers (UCSC, Ensembl), Database of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources (DECIPHER), and Database of Genomic Variants (DGV) were most often used.

In case of reported findings, the parents' genomic DNA, extracted from blood lymphocytes, was also analyzed to determine the heredity of the finding.

NGS methods

Fetal DNA was extracted either from fetal material acquired from invasive procedures prenatally or from fetal

tissues after the termination of pregnancy. NGS was performed using TruSight One (4,813 genes) or TruSight One Expanded (6,699 genes) sequencing panels (Illumina Inc.). Sequencing was carried out on a NextSeq 500 platform (Illumina). NGS was performed to probands only. Reads were aligned to the reference genome hg19 by Burrows-Wheeler Aligner (BWA) (Li & Durbin, 2009) and variants were called by Genome Analysis Toolkit (GATK) (McKenna et al., 2010) tools using BWA Enrichment v2.1. workflow on BaseSpace Onsite System (Illumina). Variants from VCF files were annotated using an in-house variant annotation pipeline involving Annovar (Wang et al., 2010), SnpSift (Cingolani et al., 2012) and GATK (McKenna et al., 2010). CNV detection was carried out using CoNIFER software (Krumm et al., 2012). All reported pathogenic, likely pathogenic, and VOUS variants were validated via Sanger sequencing in the fetus and both parents to confirm the inheritance pattern. Variants were reported according to ACMG standards (Richards et al., 2015).

2.3 | Statistical analysis

Statistical analysis was performed with STATA 16.2 software using Wilson confidence intervals for detection rates in every study group.

3 | RESULTS

3.1 | CMA analysis results

CMA was performed in 334 singleton pregnancies after cFTS or ultrasound examination with diagnosed anomaly; 184 in Group A and 150 in Group B analyses (Figure 1). A total 12 clinically significant pathogenic or likely pathogenic CNVs were found in both study groups (Table 2 and Figure 1), providing an additional diagnostic yield of 3.6% (95CI 2.07%-6.17%). Nine of these findings were in Group B, as expected, where the diagnostic yield was 6.0% (95CI 3.27%-11.29%). In Group A, the diagnostic yield of CMA was 1.6% (95CI 0.56%-4.71%). Additionally, we found 21 benign or likely benign CNVs and 11 VOUS, most of which were LCSH regions and, therefore, not reported, according to our laboratory protocol (Table S1).

3.2 | NGS analysis results

NGS analysis revealed five pathogenic variants of dysmorphic fetus, related to phenotype, among 28 selected cases. Two variants were of unknown clinical significance but fit

TABLE 1 Fetal ultrasound and autopsy findings in cases selected for NGS analysis

Case	Fetal ultrasound findings/types of anomalies on autopsy	No. of cases
Brain anomalies		9
1	Corpus callosum dysgenesis. Bilateral ventriculomegaly	
2	Corpus callosum dysgenesis. Dymorphic facial features	
3	Absence of corpus callosum. Additional spleen. Hydrops. Sandal gap	
4	Agenesis of corpus callosum	
5	Agenesis of corpus callosum. Dymorphic facial features	
6	Agenesis of corpus callosum. Lissencephaly	
7	Cerebellar hypoplasia with ventriculomegaly	
8	Holoprosencephaly	
9	Brain atrophy with hemorrhage	
Cardiac anomalies		6
10	Combined heart defect, asplenia. Malrotation of the gut	
11	Combined heart defect. Polysplenia	
12	Truncus arteriosus communis	
13	Stenosis of pulmonary artery	
14	Truncus arteriosus communis. Maternal 2q13 2,1Mb microdeletion	
15	Cardiomegaly, critical aortic stenosis	
Non-immune hydrops		4
16	Cystic hygroma and generalized hydrops	
17	Enlarged nuchal translucency and hydrops	
18	Cystic hygroma and generalized hydrops	
19	Generalized hydrops	
Multiple anomalies or syndromic suspicion		9
20	Facial cleft. Syndactyly of II-III toe. Absence of right kidney and ureter, aplasia of spleen	
21	Polycystic kidneys diagnosed at 29th week of pregnancy. Presence of ascites	
22	Unexplained anhydramnios at week 17	
23	Multiple anomalies: facial cleft, anencephaly, gastrochisis	

TABLE 1 (Continued)

Case	Fetal ultrasound findings/types of anomalies on autopsy	No. of cases
24	Large midline defect	
25	Enlarged nuchal translucency. Ectopia cordis. Gastroschisis	
26	Holoprosencephaly. Dysplastic cystic kidneys	
27	Large diaphragmatic hernia, dymorphic facial features	
28	Large spina bifida in thoracic region. Dymorphic facial features. Deformation of ribs on the right side	

with the phenotype, and there were two incidental findings also reported, based on informed consent. The additional diagnostic yield of NGS analysis in our study group was 17.9% (95CI 7.88%-35.59%). Detailed descriptions of cases with pathogenic results and results with uncertain clinical significance are provided in Table 3.

4 | DISCUSSION

We conducted a two-year study to assess the performance of molecular prenatal diagnostics in Estonia. All investigations used in this study were performed by the standard algorithm for fetal evaluation at our institution, except of CMA study Group A. The main strength of this study is its prospective structure and the combined use of several prenatal diagnostic methods. Although our study group is representative of the whole country, one of the main study weaknesses is a relatively small sample size due to our population size and annual birth rate. As of 2017, the Estonian population was 1,324,820 people and there were 13,784 births (EUROSTAT).

It has been shown that clinically relevant CNVs can be found in approximately 1.7% of fetuses, where CMA was done with indications such as advanced maternal age, anxiety, or positive serum screening without ultrasound malformations (Wapner et al., 2012). In the CMA study Group A, 184 CMA analyses were performed with widened indications, resulting in three pathogenic CNVs with clearly described associated phenotypes being identified (Table 2).

Among the pathogenic findings from Group A, the first case 15q13.3 microdeletion of 1.6 Mb was diagnosed. This region of deletion covers five genes, including *FAN1* and *TRPM1*. Phenotypically normal baby was born with normal early neonatal period. This is a highly variable syndrome associated with a higher risk to intellectual disability, epilepsy, and autistic spectrum disorders (Lowther et al., 2015). In this particular case, microdeletion was inherited from

(Continues)

TABLE 2 CMA findings in Group A and B: indications, karyotype, CMA results, interpretation, clinical significance, and outcome

Increased combined risk for trisomy 21	Karyotype	CMA result	Interpretation	Clinical significance	Pregnancy outcome (weight, length, Apgar)
Pathogenic findings in CMA Group A Pathogenic findings in CMA Group A					
1:96	46,XY	arr[GRCh37]15q13.2q13.3(30955149_32509892)x1 mat	15q13.3 microdeletion syndrome	Pathogenic	4344 g, 53 cm. Apgar 9/9
1:44	46,XX	arr(X)x1[0.2]	Mosaic Turner syndrome (10–20%)	Pathogenic	3692 g, 52 cm. Apgar 9/9
1:11	46,XY	arr[GRCh37]9q22.32q22.33(97598966_101270230)x1 dn	9q22.3 microdeletion syndrome	Pathogenic	3990 g, 54 cm. Apgar 8/9
Pathogenic findings in CMA Group B					
Complicated anamnesis	not done	arr[GRCh37]11p15.4(5228708_5343533)x1 mat	Epsilon gamma delta beta thalassemia	Pathogenic	1100 g, Apgar 1/4/5
Aortic arch pathology on ultrasound	47,XY,+idic(22)(q11.21)dn	arr[GRCh37]22q11.1q11.21(16854770_18656495)x4	Cat eye syndrome	Pathogenic	Termination
Megacystis on ultrasound	47,XY,+der(7)(7;12)(q11.21;p13.33)mat	arr[GRCh37]7p22.3q11.21(46239_63729722)x3,12p13.33(191619_916310)x3	unbalanced translocation	Pathogenic	Termination
Truncus arteriosus communis with interrupted aortic arch on ultrasound	46,XY	arr[GRCh37]16p11.2(29634212_30199805)x1	16p11.2 microdeletion syndrome	Pathogenic	Termination
Left ventricle hypoplasia on ultrasound	mos 45,X[16]/46,X,r(X)(p11.3q21.31)[4]	arr[GRCh37]Xp22.33p11.3(93118–46179305)x1, Xp11.3q21.31(46203386–89413918)x1~2, Xq21.31q28(89536405–155235833)x1	Turner syndrome mosaic variant	Pathogenic	Termination
Increased NT 8.90 mm	not done	arr[GRCh37]15q11.2(22754322_23140114)x1 mat,17p13.3(1665862_1680318)x0 mat,pat,17p13.3p13.1(783580_9127010)x2 hnz	Osteogenesis imperfecta, type VI	Pathogenic	Termination
Combined risk for trisomy 21 1:4; Increased NT 9.0 mm	46, XX	arr[GRCh37]6q23.1q23.2(130397515_134259753)x1	6q23.1q23.2 microdeletion	Likely pathogenic	Termination
Increased NT 3.6 mm	46,XY	arr[GRCh37]15q11.2(22754322_23652850)x1 mat	15q11.2 microdeletion syndrome	Likely pathogenic/ Susceptibility locus	3950 g, 50 cm. Apgar 8/9
Increased NT 3.5 mm	not done	arr[GRCh37]15q11.2(22754322_23140114)x1 mat	15q11.2 microdeletion syndrome	Likely pathogenic/ Susceptibility locus	3328 g, 48 cm. Apgar 9/9

Abbreviations: CMA, chromosomal microarray analysis; NT, nuchal translucency.

TABLE 3 Reported findings in NGS study group

Fetal findings, indication for NGS	Result	Zygosity	Pregnancy outcome	Classification	Inheritance	OMIM disease
Agensis of corpus callosum, dysmorphic features on autopsy	NM_015443.3(KANSL1):c.1652+1 G>A	Heterozygous	TOP	Pathogenic	<i>de novo</i>	Koolen-de Vries syndrome (OMIM#610443)
NT 8.0 mm; fetal hydrops	NM_002295.5(RPSA):c.413_417del p.(Ser138Cysfs*2)	Heterozygous	TOP	Pathogenic	<i>de novo</i>	Isolated congenital asplenia (OMIM#271400)
Multiple anomalies: malrotation of the bowel, cardiac anomaly on autopsy	NM_001492.5(GDF1):c.909dup p.(Val304Argfs*48)	Homozygous	TOP	Pathogenic	parents are heterozygous carriers	Right atrial isomerism, Ivemark syndrome (OMIM#208530)
Bilateral ventriculomegaly, dysgenesis of corpus callosum	NM_017791.2(FLVCR2):c.927C>A p.(Asn309Lys); NM_017791.2(FLVCR2):c.952G>T p.(Gly318Cys)	Compound heterozygous	TOP	Pathogenic	parents are heterozygous carriers	Fowler syndrome (OMIM#225790)
Facial cleft, absence of right kidney, agenesis of spleen	NM_032458.2(PHF6):c.241-1G>A	Heterozygous	TOP	Pathogenic	<i>de novo</i>	Björreson-Forssmann-Lehman syndrome (OMIM#301900)
Bilateral polycystic dysplastic kidneys	NM_000296.3(PKD1):c.8302G>A p.(Val2768Met)	Heterozygous	Livebirth	VOUS	carrier status not known	Polycystic kidney disease 1 (OMIM#173900)
VSD, truncus arteriosus communis	NM_017617.4(NOTCH1):c.680G>A p.(Cys227Tyr)	Heterozygous	TOP	VOUS	the mother is a healthy carrier	Notch receptor 1 (OMIM#190198)
Agensis of corpus callosum, additional spleen, fetal hydrops	NM_000249.2(MLH1):c.1168del p.(Glu390Asnfs*11)	Heterozygous	TOP	Incidental finding	paternal	Lynch syndrome (OMIM#120435)
Spina bifida in thoracic region, facial dysmorphism	NM_007300.3(BRCA1):c.4035del p.(Glu1346Lysfs*20)	Heterozygous	TOP	Incidental finding	mother is not a carrier ^a	Familial breast – ovarian cancer (OMIM#113705)

Abbreviations: NT, nuchal translucency; TOP, termination of pregnancy; VOUS, variant of unclear clinical significance; VSD, ventricular septum defect.

^aFather's DNA was not available for testing.

apparently healthy mother, so prognosis can be varying. This child will need a close neurobehavioral follow-up. In the second case, a low-level mosaicism (10–20%) for monosomy X was found. Ultrasound examination of the fetus was normal as well as postnatal examination of a child at the age of 1 year showed no developmental delay. Still chromosomal analysis from peripheral lymphocytes confirmed a mosaic Turner's syndrome. In this case, regular follow-up by the pediatric endocrinologist and later gynecologist is recommended, due to increased risk for infertility and endocrine disorders (Levitsky et al., 2015). The third pathogenic finding in Group A was a 3.7 Mb deletion in 9q22.32q22.33 region. This region covers 23 genes, including *FANCC*, *PTCH1*, *ERCC6L2*, *HSD17B3*, *TDRD7*, *XPA*, *FOXE1*, *NANS*, and *GPR51*. Craniosynostosis, hydrocephaly, macrosomia and intellectual disability have been described in this known 9q22.3 microdeletion syndrome (Muller et al., 2012). A baby of 3990 g. was born at term and had dysmorphic features: trigonocephaly, hypoplasia of eyebrow arches, postaxial polydactyly (rudimentary finger on the left hand), broad nasal bridge and dysmorphic ears. This baby would need a close follow-up due to the high risk of early craniosynostosis as well as early intellectual disability.

The diagnostic yield of 1.6% in Group A is, therefore, similar to the reports of other studies and reviews (Hillman et al., 2013; Vogel et al., 2018; Wapner et al., 2012). However, compared to the recently published Danish study, which reported a CNV detection rate of 2.3% in screen-positive cases after cFTS, our rate is lower (Vogel et al., 2018). This can be explained by the fact that the indication for CMA in our study was a risk greater than 1 in 100 after cFTS, whereas in the Danish study the cut-off point was a risk greater than 1 in 300.

The diagnostic yield for CMA in Group B was 6.0%, which was similar to other studies (Chong et al., 2019; Hillman et al., 2013; Vogel et al., 2018; Wapner et al., 2012). We found nine clinically significant pathogenic or likely pathogenic CNVs (Table 1). Six pregnancies were terminated due to fetal malformations and three babies were born: two cases of 15q11.2 region microdeletion and a case of 0.1 Mb deletion in 11p15.4 region. In first two 15q11.2 cases, microdeletion is inherited from apparently healthy mothers, but it is a known region for possible intellectual disability and autistic spectrum disorders (Butler, 2017), therefore these children should have a thorough follow-up by the neurologist. In second 11p15.4 deletion case, invasive diagnostics was done due to high-risk result after cFTS and familiar history. Mother has epsilon-gamma-delta-beta thalassemia due to 11p15.4 microdeletion (OMIM#141900). The same microdeletion was diagnosed in the fetus. Thus, close antenatal surveillance was conducted. By the week 30, the fetus developed signs of anemia and intrauterine blood transfusion was done. After the procedure fetus developed bradycardia and an emergency cesarean section

was performed. Hemoglobin level was 90 g/l after birth and several blood transfusions were performed. In this case CMA provided with relevant clinical information not only for postnatal life, but for antenatal surveillance as well.

In summary, we conclude that use of CMA in high-risk pregnancies after cFTS is justified and provide with relevant clinical information in about 3.6%. In the era of NIPT careful consideration should be taken in high-risk patients, whether to choose NIPT and potentially miss important microdeletions and duplications (Sotiriadis et al., 2017) or take a very small risk of miscarriage after invasive diagnostic procedure (Akolekar et al., 2015; Wulff et al., 2016), but possibly get information of a great value for prenatal and postnatal surveillance.

The incidence of VOUS among both CMA study groups was 3.3%. This number is higher than previously reported, but is very dependent on the CMA technology used, local protocols, or difficulties in interpretation during fetal life (Levy & Wapner, 2018). In this study, a SNP-array CMA platform is used. This allows us to evaluate the presence of LSCH regions, although these findings are usually not reported prenatally due to the lack of corresponding phenotypic description and the difficulties in interpreting the results. LSCH regions may rarely be reported prenatally in a case where the clinician suspects a specific recessively inherited disease based on ultrasound or post-mortem findings. Decisions about reporting or not reporting certain findings are made on a case-by-case basis (Pajusalu et al., 2015). Reporting all VOUS in the prenatal setting is challenging, but still worthwhile because some cases can change their significance over time (Stosic et al., 2018).

In the sequencing cohort, an additional diagnostic yield of 17.9% was seen after finding five clearly pathogenic findings (Table 3). All cases were selected for NGS analysis only due to ultrasound abnormalities (Table 1). This number differs between published studies. Reasons for such discrepancies include the selection of cases according to fetal anomaly, number of probands in the cohorts, and choice of NGS method used (Deden et al., 2020; Ferretti et al., 2019; Monaghan et al., 2020). In NGS studies, the additional diagnostic yields when primarily using ES range from 8.5% (Lord et al., 2019), in a large unselected cohort of 610 fetuses, to 81% (Chandler et al., 2018), in a small series of 16 fetuses strongly suspected of having skeletal dysplasia and using a targeted sequencing panel. In our previous study, NSG diagnostic performance was investigated in the adult and pediatric population in Estonia, resulting in a diagnostic yield of 26.3% (Pajusalu et al., 2018). Regarding exome and panel sequencing, similarly to CMA, challenges with reporting VOUS arise (Wert et al., 2020). In some cases, reporting VOUS variants may still be considered, for example if the known phenotype associated with the gene fits with ultrasound findings (Monaghan et al., 2020). This was the case in which *PKD1* VOUS was reported in a fetus with polycystic

kidneys and another case in which a *NOTCH1* variant was reported after ultrasound findings of a heart defect. Two pathogenic secondary findings in cancer-predisposing genes (*MLH1* and *BRCA1*) were detected in this cohort and reported back to the ordering physician for cascade screening of the family members. However, we are aware that The European Society of Human Genetics recently published their recommendations and suggested to be cautious with reporting secondary findings (Wert et al., 2020).

Our NGS results illustrate how NGS-based tests may have additional benefits beyond the scope of fetal medicine. Still, more research is needed for a better delineation of malformation groups, where NGS would give the best diagnostic result.

5 | CONCLUSION

The use of CMA in high-risk pregnancies after cFDS is justified and provides relevant clinical information in approximately 3.6% of cases. In the NGS study group, we report an added diagnostic yield of 17.9%.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

T.R. and K.Õ. conceived the study. K.R., K.A., E.-L. U, and F.Z. consulted all pregnant women and performed prenatal ultrasound and invasive procedures. M.K., P.T., and T. M.-V. performed and interpreted chromosomal microarray analyses. T.K., U.S, and S.P. performed and interpreted NGS analyses. K.R., T.R., and K.Õ. analysed all data and wrote the manuscript with input from all authors.

DATA AVAILABILITY STATEMENT

Raw data of chromosomal microarray and next-generation sequencing is available on request.

ORCID

Konstantin Ridnõi  <https://orcid.org/0000-0002-1088-5936>

Sander Pajusalu  <https://orcid.org/0000-0002-5435-0781>

Katrin Õunap  <https://orcid.org/0000-0002-4594-6364>

REFERENCES

- Akolekar, R., Beta, J., Picciarelli, G., Ogilvie, C., & D'Antonio, F. (2015). Procedure-related risk of miscarriage following amniocentesis and chorionic villus sampling: a systematic review and meta-analysis. *Ultrasound in Obstetrics & Gynecology*, *45*(1), 16–26. <https://doi.org/10.1002/uog.14636>.
- Butler, M. G. (2017). Clinical and genetic aspects of the 15q11.2 BP1-BP2 microdeletion disorder. *Journal of Intellectual Disability Research*, *61*(6), 568–579. <https://doi.org/10.1111/jir.12382>.
- Chandler, N., Best, S., Hayward, J., Faravelli, F., Mansour, S., Kivuva, E., Tapon, D., Male, A., DeVile, C., & Chitty, L. S. (2018). Rapid prenatal diagnosis using targeted exome sequencing: a cohort study to assess feasibility and potential impact on prenatal counseling and pregnancy management. *Genetics in Medicine*, *20*(11), 1430–1437. <https://doi.org/10.1038/gim.2018.30>.
- Chong, H. P., Hamilton, S., Mone, F., Cheung, K. W., Togneri, F. S., Morris, R. K., Quinlan-Jones, E., Williams, D., Allen, S., McMullan, D. J. et al (2019). Prenatal chromosomal microarray testing of fetuses with ultrasound structural anomalies: A prospective cohort study of over 1000 consecutive cases. *Prenatal Diagnosis*, *39*(12), 1064–1069. <https://doi.org/10.1002/pd.5545>.
- Cingolani, P., Patel, V. M., Coon, M., Nguyen, T., Land, S. J., Ruden, D. M., & Lu, X. (2012). Using drosophila melanogaster as a model for genotoxic chemical mutational studies with a new program. *Snpsift. Frontiers in Genetics*, *3*, 35. <https://doi.org/10.3389/fgene.2012.00035>.
- de Wert, G., Dondorp, W., Clarke, A., Dequeker, E. M. C., Cordier, C., Deans, Z., van El, C. G., Fellmann, F., Hastings, R., Hentze, S. et al (2020). Opportunistic genomic screening. Recommendations of the European Society of Human Genetics. *European Journal of Human Genetics*. <https://doi.org/10.1038/s41431-020-00758-w>.
- Deden, C., Neveling, K., Zafeiropoulou, D., Gilissen, C., Pfundt, R., Rinne, T., de Leeuw, N., Faas, B., Gardeitchik, T., Sallevelt, S. et al (2020). Rapid whole exome sequencing in pregnancies to identify the underlying genetic cause in fetuses with congenital anomalies detected by ultrasound imaging. *Prenatal Diagnosis*, *40*(8), 972–983. <https://doi.org/10.1002/pd.5717>.
- Edwards, L., & Hui, L. (2018). First and second trimester screening for fetal structural anomalies. *Seminars in Fetal & Neonatal Medicine*, *23*(2), 102–111. <https://doi.org/10.1016/j.siny.2017.11.005>.
- EUROSTAT Mean age of women at childbirth and at birth of first child in 2017. Available from <https://ec.europa.eu/eurostat/tgm/table.do?tab=table&init=1&plugin=1&language=en&pcode=tps00017>
- Ferretti, L., Mellis, R., & Chitty, L. S. (2019). Update on the use of exome sequencing in the diagnosis of fetal abnormalities. *European Journal of Medical Genetics*, *62*(8), 103663. <https://doi.org/10.1016/j.ejmg.2019.05.002>.
- Hillman, S. C., McMullan, D. J., Hall, G., Togneri, F. S., James, N., Maher, E. J., Meller, C. H., Williams, D., Wapner, R. J., Maher, E. R. et al (2013). Use of prenatal chromosomal microarray: prospective cohort study and systematic review and meta-analysis. *Ultrasound in Obstetrics & Gynecology*, *41*(6), 610–620. <https://doi.org/10.1002/uog.12464>.
- Krumm, N., Sudmant, P. H., Ko, A., O'Roak, B. J, Malig, M., Coe, B. P, Quinlan, A. R, Nickerson, D. A., & Eichler, E. E (2012). Copy number variation detection and genotyping from exome sequence data. *Genome Research*, *22*(8), 1525–1532. <https://doi.org/10.1101/gr.138115.112>.

- Levitsky, L. L., Luria, A. H., Hayes, F. J., & Lin, A. E. (2015). Turner syndrome: update on biology and management across the life span. *Current Opinion in Endocrinology, Diabetes, and Obesity*, 22(1), 65–72. <https://doi.org/10.1097/MED.000000000000128>.
- Levy, B., & Wapner, R. (2018). Prenatal diagnosis by chromosomal microarray analysis. *Fertility and Sterility*, 109(2), 201–212. <https://doi.org/10.1016/j.fertnstert.2018.01.005>.
- Li, H., & Durbin, R. (2009). Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics*, 25(14), 1754–1760. <https://doi.org/10.1093/bioinformatics/btp324>.
- Lord, J., McMullan, D. J., Eberhardt, R. Y., Rinck, G., Hamilton, S. J., Quinlan-Jones, E., Prigmore, E., Keelagher, R., Best, S. K., Carey, G. K. et al (2019). Prenatal exome sequencing analysis in fetal structural anomalies detected by ultrasonography (PAGE): a cohort study. *Lancet*, 393(10173), 747–757.
- Lowther, C., Costain, G., Stavropoulos, D. J., Melvin, R., Silversides, C. K., Andrade, D. M., So, J., Faghfoury, H., Lionel, A. C., Marshall, C. R., Scherer, S. W., & Bassett, A. S. (2015). Delineating the 15q13.3 microdeletion phenotype: a case series and comprehensive review of the literature. *Genetics in Medicine*, 17(2), 149–157. <https://doi.org/10.1038/gim.2014.83>
- McKenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernysky, A., Garimella, K., Altshuler, D., Gabriel, S., Daly, M. et al (2010). The genome analysis toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Research*, 20(9), 1297–1303. <https://doi.org/10.1101/gr.107524.110>.
- Monaghan, K. G., Leach, N. T., Pekarek, D., Prasad, P., Rose, N. C., Practice, A. P., & Guidelines, C. (2020). The use of fetal exome sequencing in prenatal diagnosis: a points to consider document of the American College of Medical Genetics and Genomics (ACMG). *Genetics in Medicine*, 22(4), 675–680. <https://doi.org/10.1038/s41436-019-0731-7>.
- Muller, E. A., Aradhya, S., Atkin, J. F., Carmany, E. P., Elliott, A. M., Chudley, A. E., Clark, R. D., Everman, D. B., Garner, S., Hall, B. D., et al. Microdeletion 9q22.3 syndrome includes metopic craniosynostosis, hydrocephalus, macrosomia, and developmental delay. *American Journal of Medical Genetics Part A*, 158A(2), 391–399. <https://doi.org/10.1002/ajmg.a.34216>.
- Muys, J., Blaumeiser, B., Jacquemyn, Y., Bandelier, C., Brison, N., Bulk, S., Chiarappa, P., Courtens, W., De Leener, A., De Rademaeker, M. et al (2018). The Belgian MicroArray Prenatal (BEMAPRE) database: A systematic nationwide repository of fetal genomic aberrations. *Prenatal Diagnosis*, 38(13), 1120–1128. <https://doi.org/10.1002/pd.5373>.
- Nowakowska, B. (2017). Clinical interpretation of copy number variants in the human genome. *Journal of Applied Genetics*, 58(4), 449–457. <https://doi.org/10.1007/s13353-017-0407-4>.
- Pajusalu, S., Kahre, T., Roomere, H., Murumets, U., Roht, L., Simenson, K., Reimand, T., & Ounap, K. (2018). Large gene panel sequencing in clinical diagnostics-results from 501 consecutive cases. *Clinical Genetics*, 93(1), 78–83. <https://doi.org/10.1111/cge.13031>.
- Pajusalu, S., Zilina, O., Yakoreva, M., Tammur, P., Kuuse, K., Molter-Vaar, T., Noukas, M., Reimand, T., & Ounap, K. (2015). The diagnostic utility of single long contiguous stretches of homozygosity in patients without parental consanguinity. *Molecular Syndromology*, 6(3), 135–140. <https://doi.org/10.1159/000438776>.
- Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., Grody, W. W., Hegde, M., Lyon, E., Spector, E. et al (2015). Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genetics in Medicine*, 17(5), 405–424. <https://doi.org/10.1038/gim.2015.30>.
- Sotiriadis, A., Papoulidis, I., Siomou, E., Papageorgiou, E., Eleftheriades, M., Papadopoulos, V., Alexiou, M., Manolagos, E., & Athanasiadis, A. (2017). Non-invasive prenatal screening versus prenatal diagnosis by array comparative genomic hybridization: a comparative retrospective study. *Prenatal Diagnosis*, 37(6), 583–592. <https://doi.org/10.1002/pd.5051>.
- Stosic, M., Levy, B., & Wapner, R. (2018). The use of chromosomal microarray analysis in prenatal diagnosis. *Obstetrics and Gynecology Clinics of North America*, 45(1), 55–68. <https://doi.org/10.1016/j.ogc.2017.10.002>.
- Ustav, E.-L., Asser, K., Muru, K., Ridnõi, K., Buts, K., Rajasalu, L., & Reimand, T. (2016). Prenatal diagnostics guideline: screening and diagnostics for chromosomal disease. Fetal ultrasound examinations. (in Estonian). Available from <https://www.ens.ee/ravijuhendid/synnitusabi>.
- Vogel, I., Petersen, O. B., Christensen, R., Hyett, J., Lou, S., & Vestergaard, E. M. (2018). Chromosomal microarray as primary diagnostic genomic tool for pregnancies at increased risk within a population-based combined first-trimester screening program. *Ultrasound in Obstetrics & Gynecology*, 51(4), 480–486. <https://doi.org/10.1002/uog.17548>.
- Wang, K., Li, M., & Hakonarson, H. (2010). ANNOVAR: Functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Research*, 38(16), e164. <https://doi.org/10.1093/nar/gkq603>.
- Wapner, R. J., Martin, C. L., Levy, B., Ballif, B. C., Eng, C. M., Zachary, J. M., Savage, M., Platt, L. D., Saltzman, D., Grobman, W. A. et al (2012). Chromosomal microarray versus karyotyping for prenatal diagnosis. *The New England Journal of Medicine*, 367(23), 2175–2184. <https://doi.org/10.1056/NEJMa1203382>.
- Wulff, C. B., Gerds, T. A., Rode, L., Ekelund, C. K., Petersen, O. B., & Tabor, A. (2016). Danish Fetal Medicine Study G: Risk of fetal loss associated with invasive testing following combined first-trimester screening for Down syndrome: a national cohort of 147 987 singleton pregnancies. *Ultrasound in Obstetrics & Gynecology*, 47(1), 38–44.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the Supporting Information section.

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