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Trio exome sequencing is highly relevant in prenatal diagnostics

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Abstract

Objective: About 3% of newborns show malformations, with about 20% of the affected having genetic causes. Clarification of genetic diseases in postnatal diagnostics was significantly improved with high-throughput sequencing, in particular through whole exome sequencing covering all protein-coding regions. Here, we aim to extend the use of this technology to prenatal diagnostics.

Method: Between 07/2018 and 10/2020, 500 pregnancies with fetal ultrasound abnormalities were analyzed after genetic counseling as part of prenatal diagnostics using WES of the fetus and parents.

Results: Molecular genetic findings could explain ultrasound abnormalities in 38% of affected fetuses. In 47% of these, disease-causing de novo variants were found. Pathogenic variants in genes with autosomal recessive or X-linked inheritance were detected in more than one-third (70/189 = 37%). The latter are associated with increased probability of recurrence, making their detection important for further pregnancies. Average time from sample receipt to report was 12 days in the recent cases.

Conclusion: Trio exome sequencing is a useful addition to prenatal diagnostics due to its high diagnostic yield and short processing time (comparable to chromosome analysis). It covers a wide spectrum of genetic changes. Comprehensive interdisciplinary counseling before and after diagnostics is indispensable.

Key points

What's already known about this topic?

- It is known that about 20% of malformations in newborns can be associated with genetic causes.

- Whole-exome sequencing, and especially trio exome sequencing, is an established and successful method in postnatal genetic diagnostics. Diagnostic yield for trio exome sequencing is around 37%.

What does this study add?
- We show that trio exome sequencing is a fast and comprehensive method in prenatal diagnostics with diagnostic yield similar to that of postnatal trio exome sequencing.
- We provide case solution rates for different phenotypic observations from 19% for abnormalities of internal organs up to 52% for skeletal malformations.

1 | INTRODUCTION

Fetal ultrasound abnormalities are found in about 3% of all pregnancies.[1] These can range from subtle abnormalities (e.g., increased nuchal translucency) to lethal conditions such as hydrops fetalis or bilateral renal agenesis. The etiology of these findings is very variable and includes exogenous as well as genetic (chromosomal, monogenic, polygenic-multifactorial) factors. Non-invasive prenatal tests (NIPT), which currently only detect numerical aberrations of chromosomes 13, 18, 21, X, and Y, as well as individual smaller copy number variants. Chorionic villus sampling or amniocentesis continues to be the starting point for genetic prenatal diagnostics.[2,3] After amniocentesis, quantitative PCR or fluorescence in situ hybridization (FISH) rapid tests are often carried out to test for the presence of trisomy 13, 18, or 21 within just a few hours.[4] In addition, microarray analyses have been shown to improve diagnosis by about 10% over conventional cytogenetics.[5] However, they are usually limited to the detection of losses or gains of genomic material larger than 20 kb. Point mutations or small copy number variations (CNV) cannot be detected with this method (Table 1).

In recent years, next-generation sequencing (NGS) has been established as a gold standard for the detection of postnatal genetic diseases in which an assignment to a specific monogenic disease is not possible due to the symptoms of the respective patient.[6] While in panel diagnostics all genes known for a disease or a group of related diseases are sequenced and analyzed in parallel, whole exome sequencing (WES) tests all protein-coding genes including the mitochondrial genome.[7,8] Only recently has high-throughput sequencing also gained importance in prenatal diagnostics.[9] Up to now, it has mostly been used for NGS panel diagnostics in the case of a corresponding ultrasound abnormality (e.g., diagnostics for RASopathies in the case of increased nuchal translucency). Prenatal panel diagnostics has the advantage that the analysis of a limited selection of well-characterized genes which minimizes the occurrence of unclear and thus difficult-to-interpret variants. However, if the causative variant is present in a gene that is not part of the panel used, no diagnosis can be made. In contrast to panel diagnostics, the benefit of prenatal exome diagnostics has so far only been investigated in a few studies with limited cohort sizes.[10] Sequence information collected during exome diagnostics is filtered bioinformatically and the remaining variants are assessed regarding their clinical relevance for the fetal ultrasound findings. In particular, the interpretation of "variants of unclear significance" (VUS) proves to be challenging. Furthermore, it is possible to detect clearly pathogenic variants in genes that are known to be unrelated to the fetal ultrasound findings (e.g., cancer predispositions) known as incidental findings. The complexity of exome diagnostics thus poses a particular challenge not only to the doctors who convey the diagnosis and for those who provide information in advance, but also to the families seeking advice.[11]

If exome diagnostics are carried out only with fetal material ("single exome analysis"), potentially pathogenic variants can be found that cannot be clearly interpreted. The clinical relevance of these variants can only be assessed by a subsequent, time-consuming comparison with the genetic information of mostly healthy parents (segregation analysis), creating additional psychological burden for those seeking advice. WES in this study was therefore tested within a framework of trio exome analysis. We bioinformatically compare coding as well as relevant intronic regions of all nuclear and mitochondrial genes of the fetus with those of the parents, filter variants, and finally selected potentially relevant variants for manual analysis. This allows us to check the origin of each variant of the fetus directly and assess whether it could be causative according to Mendelian inheritance rules. In postnatal diagnostics, there is also a significant increase in the diagnostic yield from about 21% for proband-only diagnostics to 37% in trio exome analysis.[12,13]

2 | METHODS

2.1 | Case inclusion

The presence of fetal ultrasound abnormalities was prerequisite for the indication for prenatal diagnostics. The fetal and parental DNA was usually submitted for exome diagnostics after completion of the chromosome analysis. In most cases, a negative result from NIPT, FISH rapid test, or chorionic short-time culture was obtained before exome analysis. All findings were approved by an in-house expert committee and forwarded to the submitting institution for discussion of the findings within the framework of a human genetic counseling session.

TABLE 1 Comparison of methods for prenatal genetic diagnostics

Method	Duration	Analysis targets	Resolution	Additional findings	Repeat expansions	Microdeletions and duplications	Trans-locations	Methylation defects	Uniparental disomies
NIPT	5–7 days	Aneuploidies (Chr. 13, 18, 21, X, Y); 22q11.2 microdeletion	Very large events only	-	-	(−)	-	-	-
Chromosomal analysis	2 weeks	Numerical and structural chromosomal aberrations	>5 Mb	+	-	-	+	-	-
Screening for aneuploidies (e.g., FISH, qPCR)	1 day	Aneuploidies (Chr. 13, 18, 21, X, Y)	Very large copy-number variants	-	-	-	-	-	-
Microarray	1–2 weeks	Whole-genome CNV	>50 kb	+	-	+	-	-	(−)
Sanger sequencing	1–2 weeks	Single gene exons	Point mutations	-	-	-	-	-	-
Panel sequencing	2–3 weeks	Exons of selected genes	Point mutations	-	-	-	-	-	-
Trio exome sequencing	2-3 weeks	All known exons and selected regulatory regions	Point mutations, copy-number variants	+	-	+	-	-	+

2.2 | Compliance with ethical standards

Written informed consent was obtained from each participant in the study. During the gynecological and human genetic counseling, parents were informed about the significance and limitations of (trio) exome diagnostics as well as the potential of incidental findings. All procedures performed in studies with human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its subsequent adaptations or comparable ethical standards.

2.3 | Phenotypic classification

Ultrasound findings available at the time of the sample collection (e.g. amniocentesis) were the basis for the classification of fetal phenotypes. The documented main fetal signs guided the assignment to one of the phenotype groups. The group with complex malformations contains all fetuses in which ≥2 organ systems showed ultrasound abnormalities. Further subclassification was made according to the organ system affected. "Increased nuchal translucency" was defined as a nuchal translucency measurement ≥3.0 mm without further ultrasound abnormalities. All defined phenotype groups and the respective number of affected fetuses are summarized in Table 2.

2.4 | Genotyping and classification of variants

WES was performed with fetal and parental DNA. Maternal cell contamination was excluded by comparison of the sequence data. The methodology is described in detail in the supplemental methods. After bioinformatic filtering, the remaining variants were classified according to ACMG criteria[14,15] and subjected to a medical evaluation including:

1. Variants described as disease-associated (database: HGMD©, clinvar, in-house);
2. De novo variants in disease-associated genes;
3. Potentially causative variants with very low allele frequencies in control populations;
4. Variants in disease-associated genes that can be assumed to have a functional effect;
5. Microdeletion and duplication syndromes;
6. Submicroscopic gains or losses of disease-associated genes;
7. Uniparental disomies (UPD).

2.5 | Interpretation of findings

The clinical interpretation of variants classified as relevant was carried out in context of the ultrasound findings based on published

TABLE 2 Phenotype groups and diagnostic yield

Fetal phenotype group	Definition	Total cases	Solved cases (diagnostic yield)
Skeletal malformations	Evidence of skeletal abnormalities in ultrasound, such as shortened tubular bones, multiple fractures, achondroplasia, thanatophoric dysplasia, other skeletal dysplasias	63	33 (52%)
Complex malformations	≥2 organ systems affected in ultrasound, incl. Facial dysmorphias	122	54 (44%)
Urogenital malformations	Renal agenesis, renal dysplasia, polycystic kidneys	25	11 (44%)
Brain malformations	Lissencephaly, corpus callosum agenesis, holoprosencephaly, hydrocephalus, ventrikulomegalia	79	34 (43%)
Increased nuchal transparency	Nuchal transparency >3 mm, nuchal edema, hygroma colli	72	24 (33%)
IUGR (intrauterine growth retardation)	<10th percentile	27	7 (26%)
Heart defects	Ventricular septal defect, hypoplastic left heart syndrome, tetralogy of Fallot	50	12 (24%)
Eye anomalies	Anophthalmie, cataracts	10	2 (20%)
Arthrogryposis	Arthrogryposis	10	2 (20%)
Abnormalities of internal organs	Intestinal malformations (e.g., microcolon), megacystis, malformations of the liver	21	4 (19%)
Other	For example, abnormal biochemical parameters such as PAPP-A, β-hCG; akinesia, generalized edema, harlequin ichthyosis	21	6 (29%)

cases and relevant databases (see supporting information S1 for details). All prenatal findings were subject to a separate review by a specialized team.

2.6 | Reporting of findings

Relevant variants were described in detail in the medical report sent to the submitting doctors and used in a second genetic counseling session, to communicate the results and possible consequences to the parents. The following aspects were addressed:

1. Explanation of the genetic etiology;
2. Prognosis of the course of the disease;
3. Therapy options;
4. Clinical trial options, contact to patient organizations;
5. Probability of recurrence;
6. Possibility of predictive prenatal diagnostics or pre-implantation diagnostics (PGD);
7. Possible consequences for family members;
8. Legal provisions according to applicable national law (in Germany: Gene Diagnostics Act, GenDG, and the Pregnancy Conflict Act, SchKG);
9. Consideration of the need for psychological support.

In all cases, the submitting doctors were offered a re-evaluation of the genetic data in case of changing fetal symptoms during the course of pregnancy. The possibility of a postnatal re-analysis of the sequence data was also offered for VUS.

3 | RESULTS

In this work, trio WES was carried out in 500 pregnancies with fetal ultrasound abnormalities between the 11th and 31st week of gestation. All pregnant women underwent ultrasound screening for fetal malformations in a qualified prenatal center (Degum II/III). Samples were referred from 14 centers. Following the ultrasound diagnosis, genetic counseling took place, mostly conducted by clinical geneticists. In 164 cases, the examination was carried out in parallel with the fetal karyotyping. On average, the result was available 17.8 days after receipt of the sample, with the average processing time being reduced to 11.8 days after mid-2019. The cause of the fetal abnormalities could be found by detection of pathogenic or likely pathogenic variants in 37.8% of fetuses examined (189/500) (Table S1), while 311/500 cases (62.2%) remained undiagnosed.

47.1% of the causative variants were observed to be de novo. In 29% of cases, the variants were biallelic (homozygous or compound heterozygous) in the fetus, with each parent being an asymptomatic heterozygous carrier consistent with an autosomal recessive condition. In one fetus, a causative mitochondrial variant could be detected which was also present in the mother in heteroplasmic state. Parental inheritance of a causative variant of an autosomal dominant disease was found in 17 fetuses. In 6 of these 17 fetuses, the causative variant was present in mosaic form in one of the parents, and in four cases, the parent in question also showed typical symptoms of the disease. In the remaining 11 cases, the presence of the variant in an asymptomatic parent could be explained by reduced penetrance. Of the phenotypic groups, fetuses with a skeletal phenotype showed the highest diagnostic yield (52.4%). Complex malformations could be explained in 44.3% of the cases. In fetuses with malformations of the

urogenital tract and brain, 44% and 43%, respectively, could be solved. For other phenotypic groups, the diagnostic yield was between 19% (malformations of the internal organs) and 33.3% (increased nuchal translucency). See Table 2 for a complete list including absolute cohort sizes. CNVs with genomic losses or gains (≥50 kb) were identified from WES data. CNVs assessed as causative were detected in 16 cases. By comparison with the parental data, chromosomal regions could be identified in the fetuses in which homozygous biallelic variants from only one parent could be detected (in all these cases deletions of the corresponding region was excluded). Such UPD can lead to imprinting defects if the paternal and the maternal allele are differently methylated. In the investigations carried out here, a total of four uniparental disomies were detected which could be regarded as causative for the fetal clinical picture. A summary of the results is shown in Figure 1 and Table 2.

3.1 | Variants of unclear significance

Due to the size of the examined genomic region in exome diagnostics, numerous variants arise for which a clear assessment of pathogenicity is currently not possible. These have not yet been described or characterized in the literature or are absent or very infrequent in control populations. Such variants of unclear significance were also found in the trio exome diagnostics. On average, 50–100 variants of unclear significance (according to the ACMG guidelines) were found in every case. As a rule, these variants of unclear significance were not reported in the context of prenatal diagnostics.

Nevertheless, after bioinformatic filtering, according our in-house filtering criteria (see supporting information S1), approximately five variants of unclear significance were identified in every case, which required a detailed evaluation for a possible clinical significance for the fetal phenotype.

Some reporting exceptions were made in a few cases where such a variant could explain the fetal phenotype together with a clearly pathogenic second allele in an autosomal recessive disease. Formally unclear variants were reported after a corresponding vote by our in-house ethics committee. In total, the in-house ethics committee discussed the assessment of such variants in 39 cases of which 14 were reported.

3.2 | Additional findings

In accordance with the provisions of applicable national law (German Genetic Diagnostics Act, GenDG), the scope and significance of the planned genetic diagnostics were explained during the genetic counseling, mostly carried out by clinical geneticists. In this context, it was also explicitly explained that additional findings can be found which are not related to the fetal condition. These included, for example, familial tumor predisposition syndromes, late onset diseases, or genetic conditions without therapeutic or screening interventions. On the consent form, each parent had to declare whether they would like to be informed about additional findings. In most cases the parents decided to be informed about incidental finding. Besides additional parental findings, pathogenic fetal variants were also detected which, according to current data, were not related to the ultrasound findings. The prerequisite for reporting these findings was either their relevance to a severe early childhood disease or the possibility of early therapeutic intervention for the benefit of the child. A total of nine additional fetal findings were recorded. In three cases, variants leading to a severe early childhood disease could be detected; in six other cases, therapeutic options or recommendations for action resulted, for example, the detection of a pathogenic mitochondrial variant associated with the risk of aminoglycoside-induced hearing loss. Before the additional findings were reported, they were evaluated and approved by the interdisciplinary in-house ethics committee (Figure 2, Table S2).
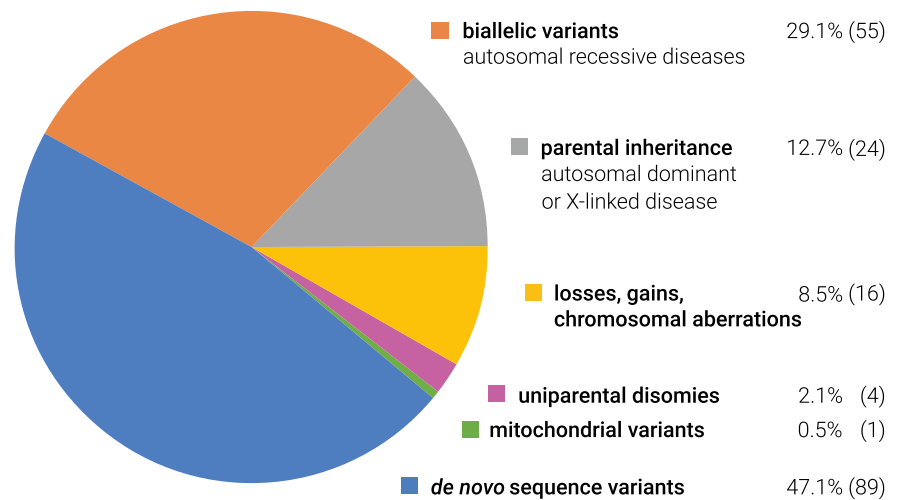

■ biallelic variants autosomal recessive diseases	29.1% (55)
■ parental inheritance autosomal dominant or X-linked disease	12.7% (24)
■ losses, gains, chromosomal aberrations	8.5% (16)
■ uniparental disomies	2.1% (4)
■ mitochondrial variants	0.5% (1)
■ *de novo* sequence variants	47.1% (89)
solved cases total	189

FIGURE 1 Solved cases by type of abnormality detected [Colour figure can be viewed at wileyonlinelibrary.com]
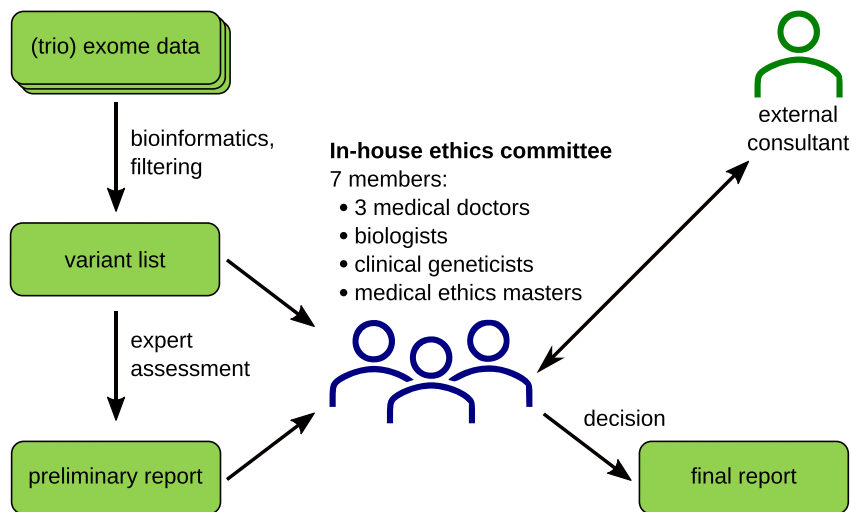
FIGURE 2 Workflow for variant reporting. Potentially relevant variants are analyzed by a specialized prenatal diagnostic team and assessed with relation to the fetal phenotype. If necessary, external experts (e.g., ultrasound diagnosticians) are consulted [Colour figure can be viewed at wileyonlinelibrary.com]

4 | DISCUSSION

Using trio-WES, the etiology of the fetal ultrasound abnormalities was detected in 189 of 500 fetuses (37.8%). This diagnostic yield was comparable to postnatal trio exome studies.[13] In 89 cases (47.1% of the solved cases), the cause was a heterozygous de novo variant, which is relevant for the probability of recurrence, as this would only be increased in the presence of a germline mosaicism in one parent. Autosomal recessive diseases were diagnosed in 55 fetuses (29.1% of the solved cases). In fewer than half of these, the pathogenic variant was present in the homozygous state, which is associated with a 25% probability of recurrence and could be consistent with parental consanguinity. A specific diagnosis enables a couple to seek PGD or an early invasive diagnostic test (e.g., chorionic villus sampling from the 11th week of gestation). A pathogenic CNV was detected in 16 fetuses (8.5% of the solved cases and 3.2% of total). Based on the WES data, both a breakpoint and size determination comparable to the resolution of a microarray examination was possible. Due to the higher sensitivity of the NGS method compared to classical Sanger sequencing,[16,17] low-level mosaicism (<5%) and heteroplasmic mitochondrial variants could also be detected. Evidence of mosaicism could be detected in seven fetuses. It is worth noting that in three cases, the pathogenic variant was present in low-level mosaic form in the maternal sample (<10%) and thus associated with a significantly increased probability of recurrence.

In addition, trio WES diagnostics may identify genetic findings that are not causally related to the fetal abnormalities but have medical relevance for one parent. Such additional findings can, for example, indicate hereditary tumor predispositions or drug intolerance (e.g., malignant hyperthermia). In the context of this work, additional findings were collected (with a corresponding declaration of consent) in 15 cases (Table S2).

This work is based on data from 500 trio exome analyses collected in the period from 07/2018 to 10/2020. During this time, optimization of the laboratory chemistry used, the sequencing equipment, and in the bioinformatic analysis of the data were achieved. The diagnostic yield is therefore based on the methodology used at the time of each analysis. The patient cohort was recruited from different prenatal centers, so the sample selection criteria were different (e.g., some of the centers sent samples only after negative microarray testing results, while other centers decided to send the samples for trio WES as first-tier testing approach). So far, genomic regions with high sequence homology, triplet repeat disorders, and methylation disorders cannot be investigated by trio exome analysis. Should these be considered for differential diagnosis, supplementary analyses are still necessary.

Recently, some publications could show that WES can be a powerful approach to identify the underlying cause in fetuses with different congenital anomalies.[18–20] The studies using prenatal trio WES are limited and often only a small number of cases were presented.[21,22] However, this study on 500 cases shows that trio WES is an effective technique in the evaluation of fetal anomalies.

5 | CONCLUSION

The present study demonstrates the importance of trio WES diagnostics for the diagnosis of fetal diseases. It allows the detection of different kinds of genetic causes in just under 2 weeks with a single test. It is therefore an important addition to the existing possibilities for genetic prenatal diagnostics (aneuploidy screening, NIPT, chromosome analysis, microarray diagnostics). The large heterogeneity of the findings obtained—pathogenic variants in 127 different genes were detected in 189 fetuses—shows the advantage of comprehensive exome diagnostics over panel diagnostics for the diagnosis of fetal ultrasound abnormalities. Before a prenatal trio exome is ordered and again, when the findings are communicated, detailed human genetic, gynecological and, if necessary, psychosocial counseling of the pregnant couple are indispensable. The prerequisites for implementing this method in prenatal diagnostics are automated laboratory processes, powerful bioinformatics, strict quality control and ongoing optimization, and a team of experienced diagnosticians in order to assess complex results in the prenatal context. WES findings may provide referring doctors with decisive information

about the fetal abnormalities as a basis for targeted counseling of the pregnant couple regarding diagnosis, prognosis, probability of recurrence, and, if necessary, diagnostic options for future pregnancies. Genetic diagnostics therefore requires interdisciplinary cooperation between experienced prenatal physicians, clinical geneticists/genetic counselors, scientists, and medical ethicists to provide pregnant couples with the best possible care in this difficult situation.

CONFLICT OF INTEREST

All authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Heinz Gabriel and Florian Battke wrote the manuscript. Dirk Korinth, Martin Ritthaler, Björn Schulte, Heinz Gabriel, and Saskia Biskup performed trio exome analysis and issued medical reports. Constantin von Kaisenberg, Max Wüstemann, Bernt Schulze, Almuth Friedrich-Freksa, Lutz Pfeiffer, Michael Entezami, Andreas Schröer, Joachim Bürger, and Holger Lebek counseled patients and referred cases for trio diagnostics. All authors read and approved the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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