Implementation of Exome Sequencing in Prenatal Diagnosis and Impact on Genetic Counseling: The Polish Experience

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Abstract: Background: Despite advances in routine prenatal cytogenetic testing, most anomalous fetuses remain without a genetic diagnosis. Exome sequencing (ES) is a molecular technique that identifies sequence variants across protein-coding regions and is now increasingly used in clinical practice. Fetal phenotypes differ from postnatal and, therefore, prenatal ES interpretation requires a large amount of data deriving from prenatal testing. The aim of our study was to present initial results of the implementation of ES to prenatal diagnosis in Polish patients and to discuss its possible clinical impact on genetic counseling. Methods: In this study we performed a retrospective review of all fetal samples referred to our laboratory for ES from cooperating centers between January 2017 and June 2021. Results: During the study period 122 fetuses were subjected to ES at our institution. There were 52 abnormal ES results: 31 in the group of fetuses with a single organ system anomaly and 21 in the group of fetuses with multisystem anomalies. The difference between groups was not statistically significant. There were 57 different pathogenic or likely pathogenic variants reported in 33 different genes. The most common were missense variants. In 17 cases the molecular diagnosis had an actual clinical impact on subsequent pregnancies or other family members. Conclusions: Exome sequencing increases the detection rate in fetuses with structural anomalies and improves genetic counseling for both the affected couple and their relatives.
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Keywords: fetal anomalies; prenatal diagnosis; ultrasound; exome sequencing; genomic variant; genotype–phenotype correlation

1. Introduction

Congenital anomalies, as defined by the World Health Organization (WHO), are structural or functional abnormalities that occur during the intrauterine life. They can be identified prenatally, at birth or any time after birth and occur in approximately 2–3% of live births and 20% of spontaneously aborted fetuses [1,2]. As the underlying etiology of congenital anomalies includes genetic and environmental factors, current guidelines recommend chromosomal microarray analysis (CMA) as a first-tier prenatal test for fetal anomalies [3]. Prenatal karyotyping can detect clinically relevant chromosomal aberrations in about 32% of fetuses with structural anomalies and CMA increases the diagnostic yield by at least 6% [4,5]. However, most of the anomalous fetuses remain without a genetic diagnosis. Exome sequencing (ES) is a powerful tool to identify sequence variants across the protein-coding regions and is increasingly used in postnatal patients. ES is also a promising method to detect the underlying genetic etiology in the fetuses with structural anomalies and normal results of routine testing [6]. The American College of Medical Genetics and Genomics (ACMG) criteria for classifying sequence variants are correlated with a clinical presentation [7]. A detected variant can be classified as pathogenic/likely pathogenic or as benign/likely benign if a particular combination of evidence of pathogenicity or benign impact is present. The remaining variants not consistent with the abovementioned criteria are classified as variants of unknown significance (VUS). In this context, a precise distinction of different fetal phenotypes with the use of Human Phenotype Ontology (HPO) terminology is essential for establishing both the diagnosis in the clinical case and general indications for prenatal ES [8,9]. Each term in the HPO describes a phenotypic abnormality and can be used to classify the anomalies in a standardized manner. The semantic clarity is necessary for communication between clinicians and scientists and for integration of biomedical human data and model organism data. There are bioinformatics tools using HPO terms (e.g., Exomiser—a JAVA program) that prioritize variants according to user-defined criteria and find potential disease-causing variants [10]. However, differences between fetal and postnatal phenotypes and limited access to additional diagnostic tests in a fetus make the interpretation of prenatal ES challenging. Therefore, a large amount of data available in public repositories and scientific reports is required to implement ES in routine prenatal diagnosis. The aim of this paper is to present the results of the first 122 prenatal ES analyses performed in our genetic department and to discuss its possible clinical impact on genetic counseling.

2. Materials and Methods

2.1. General Information

The Polish Society of Obstetricians and Gynecologists recommends at least four sono- graphic evaluations during pregnancy, which is in line with the international guidelines [11]. Patients at risk for fetal abnormality (age at delivery ≥35 years, family history of genetic or structural anomalies or abnormal ultrasound findings in current pregnancy) are offered genetic counseling and testing in terms of the National Prenatal Screening Program (NPSP) [12]. The Human Genetics Department at the Mother and Child Institute is certified by the Cytogenetic External Quality Assessment Service (CEQAS) and Polish Society of Human Genetics (pol. PTGC) and performs genetic tests for over a dozen obstetric gynecology centers that provide prenatal care from all country regions. Routine prenatal genetic
testing performed at our department comprises CMA supplemented by karyotyping based on a single flask cell culture.

We performed a retrospective analysis of the results of ES conducted at our laboratory between January 2017 and June 2021. Written informed consent for molecular testing was obtained from all individuals after appropriate genetic counseling. Clinical and family history was taken in all cases prior to testing. Participants were informed that fetal ES would be performed in terms of research; thus, only results relevant to fetal anomalies would be reported back to parents and incidental findings from the ACMG recommended list would not be evaluated nor reported [13]. All individuals consented to the use of their de-identified data for research purposes. The study design was approved by an internal bioethics committee. Indications for fetal exome testing, molecular type, reporting status and clinical significance of detected variants, mode of inheritance, genotype–phenotype correlation, pregnancy outcome and impact of the molecular diagnosis on post-test genetic counselling were analyzed. Each fetal anomaly was labeled according to HPO terminology with HP identifiers [8,9]. We arranged fetal phenotypes into 9 categories: as anomalies of the central nervous system (HP:0002011), face (HP:000271), cardiovascular system (HP:0001626), abdomen (HP:0001438), genitourinary system (HP:0000119), musculoskeletal system (HP:0033127), neural tube defects (HP:0045005), non-immune hydrops fetalis (HP:0001790) and multisystem anomalies (i.e., fetuses with anomalies of two or more categories).

2.2. Exome Sequencing Procedure

In all cases, prior to exome sequencing, a chromosomal microarray was performed using the oligonucleotide array platform CytoSure Constitutional v3 (8 × 60 k) (Oxford Gene Technology, Oxford, UK) with approximately 60,000 probes across the genome. Data were analyzed with the CytoSure Interpret Software (OGT) which provided an average resolution of 120 kb. Subsequently, DNA specimens isolated from fetal samples were sent to the external laboratory for sequencing (CeGaT, Tübingen, Germany). The sequencing procedure was performed on the NovaSeq6000 (Illumina, San Diego, CA, USA) using SureSelect Human All Exon v.6 (Agilent, Santa Clara, CA, USA) for exome capture and library preparation. The resulting raw sequence data (FASTQ format files) were post-processed on site. Short reads were mapped against the human genome reference sequence (GRCh38/hg38) using the Burrows–Wheeler Alignment (BWA) and stored as Binary Sequence Alignment Map (BAM) files. Following alignment, we used the Genome Analysis Toolkit (GATK) software for variant calling, ANNOVAR for variant annotation and the CoNIFER algorithm for CNV detection from the exome sequencing data.

2.3. Exome Variant Interpretation

Filtering of variants was conducted as a semi-rigid strategy and not precluded to reconsider any of the disregarded variants. Initial filters looked for quality control and an allele frequency, either from the open database (gnomAD < 0.01) or in-house database (<0.05; approximately 1000 postnatal samples sequenced in the same platform and processed using the same pipeline). Subsequent filtration steps were based on the molecular impact of the variant, known gene–disease correlation considering allele zygosity and clinical significance based on variant databases (ClinVar, HGMD), in silico evaluation of pathogenicity (SIFT, Polyphen-2, MutationTaster, CADD) and review of the literature. The filtering strategy was supported by the Exomiser tool in parallel to manual searching [10]. Selected variants were classified in accordance with the ACMG classification system and judged if apparently relevant to an observed fetal phenotype [7]. In each case, interpretation of the variant was discussed in a panel group of at least four participants (clinical geneticist, fetal medicine specialist, laboratory scientist and bioinformatician). Expected pathogenic or likely pathogenic variants (ACMG class 5 or 4, respectively) were verified by Sanger sequencing of the fetal DNA. Parental origin was tracked by Sanger sequencing of DNA isolated from peripheral blood of both parents. Variants of unknown significance (VUS;
ACMG class 3) were verified, tracked and reported if they were found in trans (on the other allele) with the pathogenic or likely pathogenic variant in an autosomal recessive condition that fit the fetal phenotype. After completion of the analysis, we obtained pathogenic or likely pathogenic variants that explained fetal phenotype; these variants were classified as diagnostic variants or abnormal ES results, and thus, they are referred to further in this paper. Subsequently, in cases with normal ES results we proceeded to the expanded analysis. In this step, we obtained variants in disease-related genes that potentially could explain observed fetal anomalies but did not achieve scores for pathogenic/likely pathogenic variants or variants with a strong in silico prediction of deleteriousness in genes with a limited level of evidence for disease association (also referred to as candidate genes). These variants are referred to VUS thereafter. The turnaround time of ES was around 12 weeks in each case, excluding time for the tissue culture and Sanger variants verification.

2.4. Outcome and Clinical Impact
ES results and post-test counsel were conveyed directly to patients by clinical geneticists from our center or to the referring clinical geneticist. Information on outcome was collected and divided into three categories: termination of pregnancy (TOP), livebirth and stillbirth. The impact of molecular diagnosis was subdivided into two categories: expected impact, defined as genetic risk assessment, and actual clinical impact, defined as altered reproductive management or molecular diagnosis in other family members.

2.5. Statistics
Statistical analysis was performed using STATA 12 (StataCorp LP, College Station, TX, USA). Means, medians and percentages were used to present descriptive statistics. A chi-square test was used to assess the differences between categorical data. A \( p \)-value < 0.05 was considered statistically significant.

3. Results
3.1. Cohort Characteristics
During the study period, 122 fetuses were subjected to ES at our institution. The mean maternal and gestational age at diagnosis was 31 years (range: 21–41 years) and 19.5 weeks (range: 12–35 weeks), respectively. Family history was positive in seven cases (similarly affected previous fetus, an affected parent or consanguineous parents) but no molecular diagnosis had previously been given. No exposure to any recognized teratogens or harmful environmental factors was identified in any of the patients. A detailed ultrasound examination and an invasive procedure was performed in each case (amniocentesis 114/122; 93.5%, chorionic villus sampling 6/122; 4.9%, fetal blood sampling 2/122; 1.6%). The first 11 (9.0%) samples were analyzed as trio exomes (fetus–mother–father), whereas the next cases were analyzed as fetus-only ES with targeted Sanger sequencing for detected variants in both parents. All cases were referred due to structural anomalies or fetal hydrops. We divided all cases into two groups: fetuses with a single organ system anomaly (64/122; 52.5%) and fetuses with multisystem anomalies (58/122; 47.5%).

3.2. Results by Phenotype
Overall, there were 52 (42.6%) abnormal ES results: 31 in the group of fetuses with a single organ system anomaly (31/64; 48.4%) and 21 in the group of fetuses with multisystem anomalies (21/58; 36.2%) (Tables 1 and 2). The difference between groups was not statistically significant \( X^2 (1, n = 122) = 1.86, p = 0.17 \). In a univariate logistic regression, musculoskeletal anomalies increased the odds of an abnormal ES result by 2.5 fold (OR 2.5, 95%CI 1.2–5.4; \( p = 0.016 \)) whereas cardiovascular defects decreased the odds of an abnormal ES result by 0.34 fold (OR 0.34, 95% CI 0.14–0.8; \( p = 0.014 \)).
Table 1. Diagnostic variants and variants of unknown significance detected by exome sequencing in different phenotypic groups of fetuses.

| Fetal Phenotype | Diagnostic Variants | VUS |
|-----------------|---------------------|-----|
|                 | n/N % (95% CI)      | n/N % (95% CI) |
| CNS             | 4/10 40.0 (16.7–68.8) | 2/10 20.0 (4.6–52.1) |
| Face            | 1/1 100.0 (16.8–100.0) | 0/1 0.0 (0.0–83.3) |
| Cardiovascular  | 2/12 16.7 (3.5–46.0) | 7/12 58.3 (31.9–80.7) |
| Abdomen         | 0/2 0.0 (0.0–71.0) | 1/2 50.0 (9.5–90.6) |
| Genitourinary   | 4/5 80.0 (36.0–98.0) | 0/5 0.0 (0.0–48.9) |
| Musculoskeletal | 18/29 62.1 (44.0–77.4) | 5/29 17.2 (7.1–35.0) |
| NTD             | 0/3 0.0 (0.0–61.8) | 2/3 66.7 (20.2–94.4) |
| NIHF            | 2/2 100.0 (29.0–100.0) | 0/2 0.0 (0.0–71.0) |
| Multisystem     | 21/58 36.2 (25.0–49.1) | 22/58 37.9 (26.5–50.8) |
| Total           | 52/122 42.6 (34.2–51.5) | 39/122 32.0 (24.3–40.7) |

CNS—central nervous system, NTD—neural tube defects, NIHF—nonimmune hydrops fetalis.

3.3. Results by Molecular Diagnosis

There were 57 different pathogenic or likely pathogenic variants reported in 33 different genes. The most common were missense variants (28/57; 49.1%), followed by frameshift variants (13/57; 22.8%), nonsense variants (12/57; 21.1%) and splicing variants (4/57; 7.0%); 34 variants were novel (34/57; 59.6%), whereas the remaining 23 were previously reported (23/57; 40.4%). The molecular mechanism of pathogenicity in the majority of novel variants was the same as previously reported for specific genes (31/34; 83.8%). The mechanism of pathogenicity in the three remaining novel variants was different than frequently reported, but the fetal phenotype was consistent with the literature data (Table 2 ID 10, 23 and 45). Variants of unknown significance were detected in 39 out of 70 fetuses without definite genetic diagnosis (55.7%; 1–5 VUS per fetus); 77 different variants were identified in 73 genes. Four genes were present twice (COL2A1, EVC2, GLI3 and KMT2C, presented in Table 3 with ID 63, 91, 66, 81, 76, 83, 62 and 84, respectively). The mode of inheritance was autosomal dominant (AD) in 31 cases of abnormal results (31/52; 59.6%), autosomal recessive (AR) in 18 cases (18/52; 34.6%) and X-linked (XL) in 3 cases (3/52; 5.8%). In 24 cases, based on sonographic findings, the referring clinician suggested a specific genetic diagnosis (19.7%; 24/122), which was confirmed by ES in 15 cases (62.5%; 15/24).

3.4. Outcome and Clinical Impact

The outcome of the pregnancy was known in 107 cases (87.7%; 107/122): in 54 cases the pregnancy was terminated upon parental request, because of fetal major anomalies, after appropriate counseling (TOP), 47 pregnancies ended in livebirth and 6 fetuses were stillborn. (Tables 2–4). In all 52 families with abnormal fetal ES results, the genetic risk was estimated based on the molecular diagnosis causing expected impact on individuals. Actual clinical impact was observed in 17 cases (13.9%). Reproductive management was altered in 13 families either by using highly effective contraception (n = 2) or by altered medical management in the subsequent pregnancy (n = 11), such as in vitro fertilization with preimplantation genetic diagnosis and embryo selection, CVS with targeted molecular diagnosis, ultrasound and prenatal care in the reference center. In four families, molecular diagnosis was established not only in the fetus, but also in other affected relatives.
Table 2. Sonographic findings in fetuses with abnormal exome sequencing—detailed information.

| ID | Gene | Disorder and Mode of Inheritance | Transcript | Coding Alteration | Variant Type | Variant Reporting Status | Zygosity | Inheritance | Fetal Phenotype | Outcome |
|----|------|----------------------------------|------------|-------------------|--------------|------------------------|----------|-------------|----------------|---------|
| 1  | TREDI | #225750 Aicardi–Goutières syndrome 1 (AR) | NM_033629.6 | c.[37A > C] c.[341G > A] | mr | novel | compound | mat | pat | ventriculomegaly HP:0002119, cerebral calcifications HP:0002514, abnormal cortical gyration HP:0002536 | Stillbirth |
| 2  | CEP290 | #610388 Jeune syndrome 5 (AR) | NM_025114.4 | c.[1666delA] c.[2424C > A] | fs | reported | compound | pat | mat | ventriculomegaly HP:0002119 | TOP |
| 3  | TUBA1A | #611603 Lissencephaly 3 (AD) | NM_001270399.1 | c.[985A > G] | ns | novel | heterozygous | de novo | | Livebirth |
| 4  | PQBP1 | #309500 Renpenning’s syndrome (XL) | NM_001032382.2 | c.[459_462del] | fs | reported | hemizygous | mat | | ventriculomegaly HP:0002119, abnormal cortical gyration HP:0002536 | Stillbirth |
| 5  | IRF6 | #119300 Van der Woude syndrome 1 (AD) | NM_006147.3 | c.[250G > T] | mr | reported | heterozygous | mat | | cleft upper lip HP:000204 | Livebirth |
| 6  | GATA6 | #600001 Pancreatic agenesis and congenital heart defects (AD) | NM_005257.6 | c.[1477C > T] | ns | novel | heterozygous | mat | | CAT HP:0001660 | Livebirth |
| 7  | KDM6A | #308867 Kabuki syndrome 2 (XL) | NM_021140.3 | c.[3016C > T] | ns | novel | compound | mat | | | Stillbirth |
| 8  | PKHD1 | #263200 Polycystic kidney disease 4, with or without hepatic disease (AR) | NM_138694.4 | c.[10489delC] c.[1774G > A] | fs | reported | compound | mat | | cystic kidneys HP:0000107 | Livebirth |
| 9  | PKHD1 | #263200 Polycystic kidney disease 4, with or without hepatic disease (AR) | NM_138694.4 | c.[107C > T] c.[107C > T] | ns | reported | homozygous | mat | | cystic kidneys HP:0000107 | Livebirth |
| 10 | ETFDH | #231680 Multiple acyl-CoA dehydrogenase deficiency (AR) | NM_001281738.1 | c.[1191C > A] c.[1560delA] | fs | novel | compound | mat | | cystic kidneys HP:0000107 | Livebirth |
| 11 | TMEM67 | #613500 Nephronophthisis 11 (AR) | NM_153704.6 | c.[1843T > C] c.[1843T > C] | ns | novel | compound | mat | | cystic kidneys HP:0000107 | Livebirth |
| 12 | FGFR3 | #187600 Thanatophoric dysplasia, type I (AD) | NM_000142.5 | c.[742C > T] | ns | reported | heterozygous | de novo | | short limbs HP:0009826, short ribs HP:0000773 | Livebirth |
| 13 | FGFR3 | #187600 Thanatophoric dysplasia, type I (AD) | NM_000142.5 | c.[742C > T] | ns | reported | heterozygous | de novo | | short limbs HP:0009826, short ribs HP:0000773 | Livebirth |
| ID | Gene       | Disorder and Mode of Inheritance | Transcript | Coding Alteration | Variant Type | Variant Reporting Status | Zygosity   | Inheritance | Fetal Phenotype                          | Outcome       |
|----|------------|----------------------------------|------------|------------------|--------------|-------------------------|------------|-------------|------------------------------------------|---------------|
| 14 | FGFR3      | # 187600 Thanatophoric dysplasia, type I (AD) | NM_000142.5 | c.[742C > T]      | missense     | reported                | heterozygous | de novo     | short limbs HP:0009826, short ribs HP:0000773 | Livebirth     |
| 15 | FGFR3      | # 187600 Thanatophoric dysplasia, type I (AD) | NM_000142.5 | c.[742C > T]      | missense     | reported                | heterozygous | de novo     | short limbs HP:0009826, short ribs HP:0000773 | TOP           |
| 16 | FGFR3      | # 187600 Thanatophoric dysplasia, type I (AD) | NM_000142.5 | c.[1118A > G]     | missense     | reported                | heterozygous | de novo     | short limbs HP:0009826, short ribs HP:0000773 | TOP           |
| 17 | FGFR3      | # 187601 Thanatophoric dysplasia, type II (AD) | NM_022965.4 | c.[1612A > G]     | missense     | reported                | heterozygous | de novo     | cloverleaf skull HP:0002676, short ribs HP:0000773 | Livebirth     |
| 18 | COL1A2     | # 166210 Osteogenesis imperfecta, type II (AD) | NM_000089.4 | c.[2486G > A]     | missense     | novel                   | heterozygous | pat (germinal mosaicism) | decreased skull ossification HP:0004331, short ribs HP:0000773, short limbs HP:0009826, multiple fractures HP:0005855 | TOP           |
| 19 | COL1A2     | # 166210 Osteogenesis imperfecta, type II (AD) | NC_000007.13 | g.[94053760G > A] | splicing     | novel                   | de novo     | HP:0004331, short ribs HP:0000773, short limbs HP:0009826, multiple fractures HP:0005855 | Top           |
| 20 | COL1A2     | # 166210 Osteogenesis imperfecta, type II (AD) | NM_000089.4 | c.[1739G > T]     | missense     | novel                   | hetozygous  | de novo     | decreased skull ossification HP:0004331, short ribs HP:0000773, short limbs HP:0009826, multiple fractures HP:0005855 | TOP           |
| 21 | COL2A1     | COL2A1-related skeletal dysplasia (AD) | NM_001844.5 | c.[4313_4314delinsAA] | nonsense     | novel                   | hetozygous  | de novo     | HP:0009826, short ribs HP:0000773 | TOP           |
| 22 | COL2A1     | # 259420 Osteogenesis imperfecta, type III (AD) | NM_000089.4 | c.[3269G > T]     | missense     | reported                | hetozygous  | de novo     | short limbs HP:0009826 | TOP           |
| 23 | COL2A1     | # 271700 Spondyloperipheral dysplasia (AD) | NM_001844.5 | c.[4313_4314delinsAA] | nonsense     | novel                   | hetozygous  | de novo     | short ribs HP:0000773, short limbs HP:0009826, multiple fractures HP:0005855 | Livebirth     |
| 24 | COL2A1     | # 206010 Achondrogenesis, type II or hypochondrogenesis (AD) | NM_033150.3 | c.[2815G > T]     | missense     | novel                   | hetozygous  | de novo     | short limbs HP:0009826, short ribs HP:0000773 | TOP           |
| 25 | COL2A1     | COL2A1-related skeletal dysplasia (AD) | NM_001844.5 | c.[1546G > A]     | missense     | reported                | hetozygous  | de novo     | short limbs HP:0009826 | N/A           |
| 26 | DYNC2H1    | # 613091 Short-rib thoracic dysplasia 3 with or without polydactyly (AR) | NM_001377.3 | c.[5911C > T]     | nonsense     | novel reported          | compound heterozygous | pat | short limbs HP:0009826, short ribs HP:0000773 | TOP           |
| 27 | DYNC2H1    | # 613091 Short-rib thoracic dysplasia 3 with or without polydactyly (AR) | NM_001377.3 | c.[9044A > G]     | missense     | novel                   | compound heterozygous | mat | short limbs HP:0009826 | Livebirth     |
| ID | Gene       | Disorder and Mode of Inheritance | Transcript   | Coding Alteration | Variant Type | Reporting Status | Zygosity | Inheritance | Fetal Phenotype                                                                 | Outcome |
|----|------------|---------------------------------|--------------|------------------|--------------|-----------------|----------|-------------|--------------------------------------------------------------------------------|---------|
| 28 | BMP2       | # 617877 Short stature, facial dysmorphism, and skeletal anomalies with or without cardiac anomalies (AD) | NM_001200.4  | c.[840_841insAACAC] frameshift novel | heterozygous  | pat             | micrognathia HP:0000347, Pierre Robin sequence HP:0002021, polyhydramnios HP:001561 in III trimester | Livebirth |
| 29 | NIPBL      | # 122470 Cornelia de Lange syndrome 1 (AD) | NM_133433.4  | c.[3152del] frameshift novel | heterozygous  | de novo          | micrognathia HP:0000347, Pierre Robin sequence HP:0002021, absent radius HP:0009774, absent thumb HP:0009777, hand oligodactyly HP:0001180, finger syndactyly HP:0006101 | TOP      |
| 30 | RIT1       | # 613355 Noonan syndrome 8 (AD) | NM_006912.6  | c.244T > C missense | reported      | heterozygous    | non-immune hydrops fetalis HP:0001790 | TOP      |
| 31 | PTPN11     | # 163950 Noonan syndrome 1 (AD) | NM_002834.5  | c.[188A > G] missense | reported      | heterozygous    | non-immune hydrops fetalis HP:0001790 | N/A      |
| 32 | PIEZO2     | # 248700 Marden–Walker syndrome (AD) | NM_002086.4  | c.[8056C > T] missense | novel         | heterozygous    | micrognathia HP:0000347, Dandy–Walker malformation HP:0001305, omphalocele HP:0001539, talipes HP:0001883 | N/A      |
| 33 | PIEZO2     | PIEZO2-related phenotype (AD) | NM_002086.4  | c.[140C > A] missense | novel         | heterozygous    | absence of the sacrum HP:0010305, talipes HP:0001883, AVSD HP:0006695, LAH HP:0011537, interrupted inferior vena cava with azygous continuation HP:0001671 | Livebirth |
| 34 | FGFR3      | # 187600 Thanatophoric dysplasia, type I (AD) | NM_000142.5  | c.[742C > T] missense | reported      | heterozygous    | short limbs HP:0009826, short ribs HP:0000773, frontal bossing HP:0002207, ventriculomegaly HP:0002119 | TOP      |
| 35 | EVC        | # 225500 Ellis–Van Creveld syndrome (AR) | NM_153717.2  | c.[33_34del] frameshift novel | homozygous    | mat             | short limbs HP:0009826, short ribs HP:0000773, postaxial hand polydactyly HP:0001162, AVSD HP:0006695, HAA HP:0012304 | Livebirth |
| 36 | DYNC2H1    | # 613091 Short-rib thoracic dysplasia 3 with or without polydactyly (AR) | NM_001377.3  | c.[4267C > T] [11413T > G] missense missense | compound heterozygous | mat | single ventricle heart HP:0001750, short limbs HP:0009826, talipes HP:0001883, SUA HP:0001195 | TOP      |
| 37 | TRPV4      | TRPV-related skeletal dysplasia (severe phenotype) (AD) | NM_021625.5  | c.[2187C > G] missense | novel         | heterozygous    | absence of the sacrum HP:0010305, short ribs HP:0000773, short limbs HP:0009826, talipes HP:0001883, left atrial isomerism HP:0011537 | TOP      |
| ID | Gene   | Disorder and Mode of Inheritance | Transcript         | Coding Alteration | Variant Type | Variant Reporting Status | Zygosity     | Inheritance | Fetal Phenotype                                      | Outcome |
|----|--------|---------------------------------|--------------------|------------------|--------------|-------------------------|--------------|-------------|-----------------------------------------------------|---------|
| 38 | NIPBL  | # 122470 Cornelia de Lange syndrome 1 (AD) | NM_015384.5       | c.[7319dupA]     | nonsense     | novel                   | heterozygous | de novo     | micrognathia HP:000347, short ribs HP:000773, short humerus HP:0005792, bowed humerus HP:0006557, absent forearm HP:0005632, absent hand HP:0004050, diaphragmatic hernia HP:000776, cystic kidneys HP:000107, thickened NF HP:000474 | TOP     |
| 39 | EFTUD2 | # 610536 Mandibulofacial dysostosis, Guion-Almeida type (AD) | NM_001142605.2    | c.[2593_2596del] | frameshift   | novel                   | heterozygous | de novo     | micrognathia HP:000347, Pierre Robin sequence HP:000201, basal ganglia cysts HP:0006799 | TOP     |
| 40 | MYO18B | # 61549 Klippel–Feil syndrome 4, autosomal recessive, with myopathy and facial dysmorphism (AR) | NM_001318245.2    | c.[6436C > T]    | nonsense     | novel                   | homozygous   | mat         | thoracic hemivertebrae HP:0008467, 3–4 finger syndactyly HP:0006997, positional foot deformity HP:0005656, thickened NF HP:000474 | N/A     |
| 41 | STAG2  | # 301022 Mullegama–Klein–Martinez syndrome (XL) | ENST00000218089.13 | c.[318C > G]     | nonsense     | novel                   | hemizygous   | de novo     | CAT HP:0001660, congenital diaphragmatic hernia HP:000776, spina bifida HP:0002414 | Livebirth |
| 42 | DHCR7  | # 270400 Smith–Lemli–Opitz syndrome (AR) | NM_001163817.2    | c.[452G > A]     | nonsense     | reported                | homozygous   | mat         | postaxial hand polydactyly HP:0001516, talipes bilateral HP:0001883, LALHP:0011537, TAPVR HP:0005160 | Livebirth |
| 43 | CC2D2A | # 612284 Meckel syndrome 6 (AR) | NM_001080522.2    | c.[8294delG]     | splicing     | reported                | compound     | pat         | postaxial hand polydactyly HP:0001162, postaxial foot polydactyly HP:0001830, encephalocoele HP:0002084, cystic kidneys HP:000107 | TOP     |
| 44 | CEP290 | # 610188 Joubert syndrome 5 (AR) | NM_025114.4       | c.[1992del]      | frameshift   | reported                | compound     | pat         | cystic kidneys HP:0003107, aplasia of the cerebellar vermis HP:0006817 | Livebirth |
| 45 | NEK9   | # 617022 Lethal congenital contracture syndrome 10 (AR) | NM_003116.6       | c.[115_116dup]   | frameshift   | missense                | compound     | pat         | fetal akinesia sequence HP:0001989, abnormally shaped abdomen (protruberant abdomen) HP:0001538 | TOP     |
| 46 | BICD2  | # 618291 Spinal muscular atrophy, lower extremity-predominant, 2B (AD) | NM_001003800.2    | c.[2100C > A]    | missense     | novel                   | heterozygous | de novo     | fetal akinesia sequence HP:0001989, choroid plexus cyst HP:0002190, hydrops fetalis HP:0007899 | TOP     |
Table 2. Cont.

| ID | Gene | Disorder and Mode of Inheritance | Transcript | Coding Alteration | Variant Type | Variant Reporting Status | Zygosity | Inheritance | Fetal Phenotype | Outcome |
|----|------|----------------------------------|------------|-------------------|--------------|------------------------|----------|-------------|----------------|---------|
| 47 | ACTA1 | # 161800 Nemaline myopathy 3, autosomal dominant or recessive (AD) | NM_001100.4 | c.[478G > A] missense | reported | heterozygous | de novo | overlapping fingers bilateral HP:0010557, knees fixed extension bilateral HP:0005085, talipes bilateral HP:0001883, hydrdops fetalis HP:0001789, polyhydramnios HP:0001561 | Stillbirth |
| 48 | GBE1 | # 232500 Glycogen storage disease IV (AR) | NC_000003.11 | g.[81627070_81627073del] g.[81698005A > G] splicing splicing | novel reported | compound heterozygous | pat mat | fetal akinesia sequence HP:0001989, antecubital pterygia bilateral HP:0009760, hydrdops fetalis HP:0001789 | TOP |
| 49 | PHGDH | # 256520 Neu–Laxova syndrome 1 (AR) | NM_006623.4 | c.[1447_1462del] frameshift frameshift | novel | homozygous | pat mat | fetal akinesia sequence HP:0001989, disproportinate short trunk HP:0003521, lissencephaly HP:0001339, cerebellar hypoplasia HP:0001321, cleft upper lip HP:000204, microphthalmia HP:0000568 | TOP |
| 50 | MAGEL2 | # 615547 Schaaf–Yang syndrome (AD) | NM_019066.5 | c.[1853del] frameshift | novel | heterozygous | de novo | fetal akinesia sequence HP:0001989, hydrdops fetalis HP:0001789 | TOP |
| 51 | AGRN | # 615120 Myasthenic syndrome, congenital, 8, with pre- and postsynaptic defects (AR) | NM_198576.4 | c.[4657delT] 1p36.33(961,395-1,109,913)x1 frameshift deletion | novel | compound heterozygous | mat pat | fetal akinesia sequence HP:0001989, hydrdops fetalis HP:0001789 | Stillbirth |
| 52 | RIT1 | # 61355 Noonan syndrome 8 (AD) | NM_001256820.2 | c.[162G > T] missense | reported | heterozygous | de novo | VSD HP:0006695, ileus HP:0002595, cystic hygroma HP:0000476 | TOP |

AVSD—atrioventricular canal defect, CAT—common arterial trunk (truncus arteriosus), CNS—central nervous system, HAA—hypoplastic aortic arch, HLHS—hypoplastic left heart, LAI—left atrial isomerism, NF—nuchal fold, SUA—single umbilical artery, TAPVR—total anomalous pulmonary venous return, VSD—ventricular septal defect, N/A—not available; names of the genes are given according to HUGO Gene Nomenclature Committee (HGNC), names of the disorders are given according to Online Mendelian Inheritance in Man database (OMIM), phenotypic descriptions with HP identifiers are given according to Human Phenotype Ontology (HPO); mode of inheritance: AD-autosomal dominant, AR—autosomal recessive, XL—X-linked.

Table 3. Sonographic findings in fetuses with VUSs detected in exome sequencing—detailed information.

| ID | Fetal Phenotype | Gene | Outcome |
|----|----------------|------|---------|
| 53 | ventriculomegaly HP:0002119 | LAMA1 | TOP |
| 54 | cerebellar vermis hypoplasia HP:0001320 | KMT2D, MED13, COG8, ARHGEF2 | N/A |
| 55 | VSD HP:0001629 | ARID1B | Livebirth |
| ID  | Fetal Phenotype                              | Gene                                | Outcome |
|-----|---------------------------------------------|-------------------------------------|---------|
| 56  | HLHS HP:0004383                             | RIT1, CDK13, CEP41, TGFBR1          | TOP     |
| 57  | TGA HP:0001669                              | CHD3, DNAH1, ESCO2                   | Livebirth |
| 58  | single ventricle heart HP:0001750, TGA HP:0001669 | ABL1, AFF4                           | Livebirth |
| 59  | pulmonary valve atresia HP:0010882           | TNXB, PACS1                          | Livebirth |
| 60  | pulmonary valve atresia HP:0010882           | GATAD2B                              | TOP     |
| 61  | mitral regurgitation HP:0001653, cardiomegaly HP:0001640 | TTN                                  | TOP     |

**Abdomen**

| ID  | Fetal Phenotype                              | Gene                                | Outcome |
|-----|---------------------------------------------|-------------------------------------|---------|
| 62  | heterotaxy HP:0030853                        | TENT5A, KMT2C, PIEZO1                | Livebirth |

**Musculoskeletal**

| ID  | Fetal Phenotype                              | Gene                                | Outcome |
|-----|---------------------------------------------|-------------------------------------|---------|
| 63  | short limbs HP:0009826                      | COL2A1, LTBP3                        | N/A     |
| 64  | short limbs HP:0009826                      | PDE4D, COL11A2, TWIST1               | N/A     |
| 65  | short limbs HP:0009826, talipes HP:0001883  | COL10A1, TGFBR1, CLCN7              | N/A     |
| 66  | femoral bowing HP:0002980, talipes HP:0001883 | LAMA5, ZNF407, PYGM, EVC2          | N/A     |
| 67  | iniencephaly HP:0010674, arthrogryposis-like hand anomaly HP:0005612, talipes HP:0001883 | LG4                                  | TOP     |

**NTD**

| ID  | Fetal Phenotype                              | Gene                                | Outcome |
|-----|---------------------------------------------|-------------------------------------|---------|
| 68  | spina bifida HP:0002414                     | ACAN, ERCC6, SON                    | TOP     |
| 69  | spina bifida HP:0002414                     | ACAN, PLEKHM1                       | TOP     |

**Multisystem**

| ID  | Fetal Phenotype                              | Gene                                | Outcome |
|-----|---------------------------------------------|-------------------------------------|---------|
| 70  | ventriculomegaly HP:0002119, absent septum pellucidum HP:0001331, cleft upper lip HP:0000204 | NEK1, DYNC2H1                      | Livebirth |
| 71  | ventriculomegaly HP:0002119, cerebellar hypoplasia HP:0001321, cerebellar vermis hypoplasia HP:0001320, Dandy–Walker malformation HP:0001305, abnormal cortical gyration HP:0002536, short ribs HP:0000773, short limbs HP:0009826 | SMARCB2                             | N/A     |
| 72  | ventriculomegaly HP:0002119, cerebellar hypoplasia HP:0001321, cerebellar vermis hypoplasia HP:0001320, DORV HP:0001719, VSD HP:0001629, radial club hand HP:0004059 | ANKRD11                              | TOP     |
| 73  | ventriculomegaly HP:0002119, congenital diaphragmatic hernia HP:0000776, pyelectasis HP:0001945, talipes HP:0001883 | CTC1, EBF4                          | Livebirth |
Table 3. Cont.

| ID  | Fetal Phenotype                                                                 | Gene                                                                 | Outcome  |
|-----|---------------------------------------------------------------------------------|----------------------------------------------------------------------|---------|
| 74  | ventriculomegaly HP:0002119, microphthalmia HP:0000568, VSD HP:0001629           | ZDHHC9, GPC3, CNKSR2                                                | N/A     |
| 75  | ventriculomegaly HP:0002119, microphthalmia HP:0000568, DORV HP:0001719         | MYCN, MED12L, ZFPM2, NOTCH1, EP300                                   | N/A     |
| 76  | abnormal cortical gyration HP:0002536, microphthalmia HP:0000568, cataract HP:0000518, abnormal sex determination HP:0012244 (phenotype female, genotype male) | GLI3                                               | Livebirth |
| 77  | agenesis of corpus callosum HP:0001274, cerebellar vermis hypoplasia HP:0001320, knees fixed extension HP:0005085, overlapping fingers HP:0010557 | NALCN, MAPK8IP3, DST, KIF1A                                 | Livebirth |
| 78  | anencephaly HP:0002323, spina bifida HP:0002414, abnormal heart morphology HP:0001627 | NCK2, BOC                                     | Livebirth |
| 79  | cleft upper lip HP:0000204, hypoplasia of right ventricle HP:0004762, VSD HP:0001629 | KCNH2, MEIS2                            | TOP     |
| 80  | CAT HP:0001660, VSD HP:0001629, preaxial foot polydactyly HP:0001841, SUA HP:0001195 | MKKS                                               | Livebirth |
| 81  | DORV HP:0001719, absent radius HP:0003974                                       | EVC2                                          | TOP     |
| 82  | AVSD HP:0006695, hand polydactyly HP:0001161, foot polydactyly HP:0001829, unilateral renal agenesis HP:000122 | SMO                                               | N/A     |
| 83  | CAT HP:0001660, short ribs HP:0000773, scoliosis HP:0002650, preaxial foot polydactyly HP:0001841, unilateral renal agenesis HP:000122, cystic hygroma HP:0000476 | GLI3, DLG5                          | Livebirth |
| 84  | omphalocele HP:0001539, radial club hand HP:0004059, cystic hygroma HP:0000476 | KMT2C                                      | Livebirth |
| 85  | micrognathia HP:0000347, absent forearm HP:0005632, absent hand HP:0004059, radial club hand HP:0004059, VSD HP:0001629, diaphragmatic hernia HP:0000776 | COLEC11                                 | Stillbirth |
| 86  | micrognathia HP:0000347, cystic kidneys HP:0001017, cystic hygroma HP:0000476 | HESX1                                      | TOP     |
| 87  | micrognathia HP:0000347, fetal akinesia sequence HP:0001989, non-immune hydrops fetalis HP:0001790 | ACADVL                                  | TOP     |
| 88  | fetal akinesia sequence HP:0001989, 2–3 finger syndactyly HP:0001233          | SYNE2, COL6A1                               | TOP     |
| 89  | LBWC HP:N/A                                                                    | CEP120                                      | TOP     |
| 90  | LBWC HP:N/A                                                                    | FLNB                                        | TOP     |
| 91  | LBWC HP:N/A                                                                    | COL2A1                                      | TOP     |

AVSD—atrioventricular canal defect, CAT—common arterial trunk (truncus arteriosus), CNS—central nervous system, DORV—double outlet right ventricle, HLHS—hypoplastic left heart, LBWC—limb-body wall complex, N/A—not available, NTD—neural tube defect, SUA—single umbilical artery, TGA—transposition of great arteries, VSD—ventricular septal defect; names of the genes are given according to HUGO Gene Nomenclature Committee (HGNC), names of the disorders are given according to Online Mendelian Inheritance in Man database (OMIM), phenotypic descriptions with HP identifiers are given according to Human Phenotype Ontology (HPO).
Table 4. Sonographic findings in fetuses with normal ES detected in exome sequencing—detailed information.

| ID | Fetal Phenotype                                                                 | Outcome |
|----|--------------------------------------------------------------------------------|---------|
|    | **CNS**                                                                         |         |
| 92 | cerebellar vermis hypoplasia HP:0001320, ventriculomegaly HP:0002119             | Livebirth|
| 93 | ventriculomegaly HP:0002119                                                    | TOP     |
| 94 | absent septum pellucidum HP:0001331                                            | Livebirth|
| 95 | ventriculomegaly HP:0002119                                                    | Livebirth|
|    | **Cardiovascular**                                                             |         |
| 96 | HLHS HP:0004383                                                                 | TOP     |
| 97 | single ventricle heart HP:0001750, CAT HP:0001660                                | TOP     |
| 98 | lymphangioma HP:0100764                                                         | Livebirth|
|    | **Abdomen**                                                                     |         |
| 99 | omphalocele HP:0001539                                                          | Livebirth|
|    | **Genitourinary**                                                              |         |
| 100| cystic kidneys HP:0000107                                                        | Livebirth|
|    | **Musculoskeletal**                                                            |         |
| 101| absent tibia HP:0009556, fibular aplasia HP:0002990, absent foot HP:0011301, radial club hand HP:0004059, hand monodactyly HP:0004058 | TOP     |
| 102| aplasia of the fingers HP:0009380 in one hand, 2–3 finger syndactyly HP:0001233 and triphalangeal thumb HP:0001199 in the other hand | Livebirth|
| 103| skull bone defect with intact skin HP:0001362, micrognathia HP:0000347, proptosis HP:0000520 | Livebirth|
| 104| short limbs HP:0009826, craniostenosis HP:0001363                               | Livebirth|
| 105| absent radius HP:0003974, hand oligodactyly HP:0001180                         | TOP     |
| 106| short limbs HP:0009826                                                          | N/A     |
|    | **NTD**                                                                         |         |
| 107| spina bifida HP:0002414                                                         | Livebirth|
|    | **Multisystem**                                                                |         |
| 108| ventriculomegaly HP:0002119, tetralogy of Fallot HP:0001636                    | TOP     |
| 109| ventriculomegaly HP:0002119, ileus HP:0002595, hydronephrosis HP:0000126, unilateral renal agenesis HP:0000122, PRUV HP:N/A, SUA HP:0001195 | Livebirth|
| 110| spina bifida HP:0002414, congenital diaphragmatic hernia HP:0000776             | TOP     |
### Table 4. Cont.

| ID  | Fetal Phenotype                                                                 | Outcome |
|-----|---------------------------------------------------------------------------------|---------|
| 111 | LBWC HP:N/A                                                                      | TOP     |
| 112 | encephalocele HP:0002084, left atrial isomerism HP:0011537                       | TOP     |
| 113 | ventriculomegaly HP:0002119, hypoplasia of the cerebellum HP:0007360, tetralogy of Fallot HP:0001636 | TOP     |
| 114 | single ventricle heart HP:0001750, heterotaxy HP:0030853, SUA HP:0001195         | TOP     |
| 115 | micrognathia HP:0000347, absence of the sacrum HP:0010305, short limbs HP:0009826, arthrogryposis-like hand anomaly HP:0005612, talipes HP:0001883, foot polydactyly (8 toes) HP:0001829, ventriculomegaly HP:0002119, CAT HP:0001660, thickened NF HP:0000474 | Stillbirth |
| 116 | knees fixed extension HP:0005085, talipes HP:0001883, ileus HP:0002595, ascites HP:0001791 | TOP     |
| 117 | fused cervical vertebrae HP:0002949, posterior fossa cyst HP:0007291             | Livebirth |
| 118 | talipes HP:0001883, ileus HP:0002595                                             | Livebirth |
| 119 | LBWC HP:N/A                                                                      | TOP     |
| 120 | congenital diaphragmatic hernia HP:0000776                                       | Livebirth |
| 121 | ventriculomegaly HP:0002119, cystic kidneys HP:0000107, thickened NF HP:0000474 | N/A     |
| 122 | cardiomegaly HP:0001640, short limbs HP:0009826, hydrops fetalis HP:0001789, polyhydramnios HP:0001561 | Livebirth |

CAT—common arterial trunk (truncus arteriosus), CNS—central nervous system, HLHS—hypoplastic left heart, LBWC—limb-body wall complex, N/A—not available, NF—nuchal fold, NTD—neural tube defect, PRUV—persistent right umbilical artery, SUA—single umbilical artery; phenotypic descriptions with HP identifiers are given according to Human Phenotype Ontology (HPO).
4. Discussion

4.1. Diagnostic Yield of Prenatal ES

Previous studies report a wide range of the added value of prenatal ES, ranging between 6 and 80% [14,15]. The discrepancy between various studies is most probably due to different study populations, inclusion criteria and sample size. Two large prospective studies on 610 and 234 fetuses with various sonographic anomalies, including isolated, increased NT, indicated a diagnostic yield of ES of 8.5% and 10%, respectively [16,17]. In contrast, Fu et al. reported a diagnostic yield of ES of 24% in a group of 191 fetuses with structural malformations; however, they excluded fetuses with isolated, large NT or cystic hygroma [18]. In our cohort, the added value of ES was even higher and reached 42.6%, similar to 39.4% reported by He et al. [19]. This may be due to a relatively high proportion of cases with skeletal anomalies included and can be considered as selection bias. We found a high diagnostic rate of 62.1% in fetuses with musculoskeletal system abnormalities, which is consistent with the data from other authors [16–21]. Interestingly, in our cohort cardiac defects significantly decreased the odds of an abnormal ES result. The proportion of detected pathogenic or likely pathogenic variants in fetuses with isolated cardiac defects was 16.7%, slightly higher than 11.5% reported in a large prospective cohort study and systematic review [22]. The diagnostic yield in both isolated, specific anomalies and recognizable multisystem sets of anomalies is probably more valuable for clinical practice than the overall diagnostic yield in heterogenous cohorts.

4.2. Interpretation of Detected Variants

The process of reaching the ES result is based on discarding variants that are unlikely to be responsible for the disease and leaving a variant or variants correlated with the phenotype. In our research, Exomiser was helpful in the detection of likely causative variants, but the final decision relied on man. The interpretation of VUS in a prenatal setting presented a particular challenge [23,24]. In three cases of limb-body wall complex (LBWC), the condition where severe anomalies are not compatible with postnatal life, the significance of variants detected in CEP120, FLNB and COL2A1 genes was unknown (Table 3 ID 89, 90 and 91). Complexity of the disorder, lack of the specified HPO term for LBWC and absence of postnatal patients with LBWC caused problems in sequence variants interpretation. Potential risks of both overdiagnosing the variant as pathogenic and misdiagnosing as a benign finding should be considered. Therefore, in selected cases, a consensus decision made in a multidisciplinary panel hastened the diagnosis. Lefebvre et al. described a blind to phenotype, genotype-first approach that we do not consider appropriate—without knowing the phenotype, the variants cannot be interpreted [25].

4.3. Molecular Mechanism, Inheritance Mode and Their Clinical Impact

The most common molecular changes in our cohort were missense variants, which is in line with other studies [16–18,26]. In the majority of cases, the mechanism of pathogenicity was in accordance with previous reports. In three cases, however, the mechanism was different and a detailed sonographic description enabled a correct diagnosis (Table 2 ID 10, 23 and 45). For instance, two novel heterozygous variants (missense and frameshift) in the NEK9 gene were found in a fetus, which presented with micrognathia, akinesia, an abnormally shaped abdomen and an increased level of free β-hCG (14.1 MoM) at 12 weeks of gestation (Table 2 ID 45). Pathogenic missense variants in the NEK9 gene have previously been reported in individuals with arthrogryposis, avascular necrosis of the femoral head and cardiac defects, which was not consistent with the anomalies detected in our case. However, a literature review revealed a report of two related families, in which five offspring were homozygous for a nonsense variant in NEK9 exactly phenotypically matching our case [27]. To our knowledge, this is the first report of the lethal congenital contracture syndrome (OMIM # 617022) caused by the NEK9 variant and diagnosed in the first trimester.
The mode of inheritance in our cohort was similar to that described in other studies [16–23,25]. The majority of diagnostic variants were de novo autosomal dominant, most fetuses with autosomal recessive variants were compound heterozygotes and X-linked inheritance was the least common.

By having information on a detected diagnostic variant and its inheritance trait, we were able to estimate the recurrence risk in affected couples. Furthermore, we observed that 13 families benefited from the exome results to act according to their beliefs and make conscious decisions. As noticed by several authors, the introduction of prenatal ES is important in a wide context of genetic counseling [26,28–32]. It can offer considerable advantages for both the couple of the affected fetus and other family members. The turnaround time in our study was around 12 weeks; thus, the results did not have a direct impact on clinical management in current pregnancies. However, by gaining experience the turnaround time can be reduced, which is essential for the decision making in ongoing pregnancies.

5. Conclusions

To conclude, exome sequencing increases the detection rate in fetuses with structural anomalies and improves genetic counseling. Establishing a molecular diagnosis has clinical impact on subsequent pregnancies, as well as other family members.

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