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The Importance of Prenatal WES Testing in the Romanian Population

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Abstract: Objective: We aimed to assess Whole Exome Sequencing's (WES) utility in Romanian prenatal care, emphasizing its diagnostic capabilities compared to molecular karyotyping, specifically for cases with negative results and consanguinity. Methods: Initially, we identified pregnancies with abnormal ultrasounds unrelated to known syndromes. Subsequently, we performed SNP-array testing, yielding negative results in all cases except one (Case 9). We then applied WES, utilizing Massive Parallel Sequencing on the NovaSeq 6000 platform (average coverage >100X, read length: 2x100bp) with library preparation using the Twist Human Core Exome kit RefSeq & Mitochondrial panel (Twist Bioscience). Bioinformatic analysis involved direct comparison to the human reference sequence (hg38). Results: Among our 10-patient cohort, we achieved a 50% diagnostic rate. After receiving results, two couples chose pregnancy termination, five had uneventful births and continue to be monitored, but sadly, one pregnancy ended in stillbirth. Additionally, we identified three incidental findings that enhanced patient and at-risk member management. Conclusion: This article details ten prenatal cases subjected to WES, highlighting its superior diagnostic performance compared to SNP-array in our specific context. We emphasize the advantages of WES in prenatal diagnostics while acknowledging the need for further investigations to comprehensively evaluate its diagnostic utility in the Romanian population.

Keywords: prenatal; WES; sequencing; exome; fetal; malformations

1. Introduction

The prevalence of congenital anomalies in the general population is approximately 3%-5% [1]. Since the first report of its use in obstetrics, ultrasound has become an important tool for the detection of fetal structural defects, identifying them in 2-3% of the pregnancies [2].

More than 75-80% of fetal anomalies appear in the first 3 months of gestation. Therefore, a good visualization of the fetus in this stadium allows early detection of structural anomalies [6-8]. The detection rates of the major structural anomalies for ultrasound examination in the first and second trimester are from 12% to 44% and from 20% to 87% respectively [9-13].

In developed countries, the number of referrals for first-trimester ultrasound (11 weeks to 13 + 6 weeks) has increased, while second-trimester ultrasound is considered the gold standard for detecting structural abnormalities. These patients are referred to chorionic villus biopsy or amniocentesis with karyotype and/or fetal molecular karyotype testing. The result of the classic karyotype identifies a diagnosis in 7-10% of pregnancies and the comparative genomic analysis adds approximately 6% to the etiology of fetal anomalies [6]. However, most cases remain without a genetic diagnosis.

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Whole exome sequencing (WES), is currently clinically available and focuses only on exons or protein-coding regions of the genome. Exons represent 1.5-2% of the total genomic DNA, comprising approximately 22,500 genes.

2. Materials and Methods

After selecting pregnancies with abnormal ultrasound findings, the testing algorithm was: first SNP karyotyping and then WES, for the negative ones, using Massive Parallel Sequencing of the whole coding region of the human genome on Next Generation Sequencing Platform, NovaSeq 6000 (average coverage >100X, read length: 2x100bp). The Twist Human Core Exome kit RefSeq & Mitochondrial panel (Twist Bioscience) was used for the library preparation. Bioinformatic analysis was performed by direct comparison of the genome of the test sample with the human reference sequence (hg38).

Limitations of prenatal WES: WES does not detect small copy number variations, it does not detect aneuploidy, polyploidy, translocations, trinucleotide repeats or low levels of mosaicism. There are also regions in the genome with low coverage by WES, in particular CpG-rich regions that will not be properly sequenced. The execution time for prenatal WES represents an important limitation [4,5,6].

3. Results

We hereby describe 10 cases of prenatal WES testing for pregnant mothers from Romanian population, between 2022 and 2023 from a private laboratory in Bucharest, Romania.

Table 1. The 10 cases of prenatal WES testing for pregnant mothers.

Case no.	*Phenotype*	*Gestational age*	*Reason for WES testing referral*	*Fetal WES*	*Pregnancy outcome*	*Incidental/secondary findings*
1	Fetal sinusal bradycardia, supraventricular extrasystoles	22 weeks	Negative SNP array Known genes linked to fetal phenotype	Negative	Stillbirth	PALB2:c.93dupA(p.Leu32thrfsTer11) – inherited from the mother
2	Aberrant right subclavicular artery, rocker bottom feet, fetal hydrops, fetal ascites	20 weeks	Negative SNP array	LMX1B:c.718G>A (p.Val240Ile) Focal segmental glomerulosclerosis 10 Nail-patella syndrome	Born, affirmatively without symptoms at the age of 1 y.o.	-
3	Right aortic arch, severe hydronephrosis, caudal regression syndrome, mesoaxial hand polydactyly	18 weeks	Negative SNP array Clinical suspicion of VACTERL syndrome	HOXD13:c.820C>T (p.Arg274Ter) ?Brachydactyly-syndactyly syndrome Brachydactyly, type D Brachydactyly, type E Syndactyly, type V Synpolydactyly 1	Couple decided termination of pregnancy	-
4	Mega Cisterna Magna, ventriculomegaly	22 weeks	Negative SNP array	ADNP:c.1612G>A (p.Glu538Lys) Helsmoortel-van der Aa syndrome	Ongoing pregnancy	-

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				De novo		
5	Clubfeet, microretrognathia, arachnodactyly, agenesis of the corpus callosum, intrauterine growth restriction	24 weeks	Negative SNP array	TGFBR1: c.734A>C (p.Glu245Ala) Loeys Dietz syndrome	Born, affirmatively without symptoms at the age of 1 y.o.	HBB: c.-151C>T Beta thalassemia Inherited from the mother
6	Corpus Callosum agenesis Oligohydramnios	23 weeks	Consanguinity Negative SNP array	Negative	Born, affirmatively without symptoms at the age of 2 y.o.	BRCA2: c.793+1G>A Susceptibility to breast-ovarian cancer, pancreatic cancer, prostate cancer Inherited from mother
7	Intracardiac echogenic focus, pyelectasis. Long QT syndrome of the father, family history of sudden cardiac death, arrhythmias due to affected father (without genetic diagnosis)	19 weeks	Negative SNP array	KCNQ1:c.605-28A>G Long QT syndrome 1 Susceptibility to Short QT syndrome 2 Atrial fibrillation, familial, 3 Inherited from the father	Born, affirmatively without symptoms at the age of 1 y.o.	-
8	Right aortic arch	17 weeks	Negative SNP array	Negative	Born, affirmatively without symptoms at the age of 1 y.o.	-
9	Sexual ambiguity on ultrasound morphology. Intrauterine growth restriction, hypospadias, polyhydramnios.	20 weeks	SNP array: arr[GRCh38]12p13.33p11.22(148769_30138756)x2 hmz, 12q21.31q24.22(84757938_117685540)x2 hmz	Negative	Couple decided termination of pregnancy	-
10	Borderline bilateral ventriculomegaly, suspicion of hydrocephaly with Sylvian stenosis	19 weeks	Negative SNP array	Negative LAMB1:c.3499C>T (p.Arg1167Ter) Lissencephaly 5 (AR) PTPN23:c.2248C>A (p.Pro750Thr) Neurodevelopmental	Ongoing pregnancy	-

		disorder and structural brain anomalies with or without seizures and spasticity (AR)	

1. In the first case, we present a 26 years old pregnant woman for whom the second trimester ultrasound revealed fetal sinusal bradycardia, supraventricular extrasystoles. There are a lot of genes associated with channelopathies, which increased the chance of diagnosis using whole exome sequencing. The purpose of the test was to detect for the possible presence of point mutations (nucleotide substitutions & deletion/insertion of some base pairs) in 62 genes associated with cardiac arrhythmias. The result was negative for pathogenic or likely pathogenic variants in channelopathy genes but with incidental findings of a pathogenic variant in the PALB2 gene (inherited from the mother).

Familial history was negative for cancers. Following the genetic counseling, the couple decided to keep the pregnancy and also an adequate monitoring plan was made for the mother and relatives at risk.

The c.93dupA variant inserts 1 nucleotide in exon 2 of the PALB2 gene, creating a frameshift and premature translation stop signal, resulting in an absent or non-functional protein. Loss-of-function variants in the PALB2 gene are known to be pathogenic (PMID: 17200668, 24136930, 25099575). This variant is not found in the gnomAD database. This variant has been detected in multiple individuals with a personal and/or family history of breast cancer (PMID: 25452441, 28008555, 27878467, 29785153, 31159747)

Women with a pathogenic or likely pathogenic variant in the PALB2 gene have a risk of breast cancer that is estimated to be 9.47 times higher than the average. Particularly, women carriers of PALB2 pathogenic variants have a 14% risk of developing breast cancer by age 50 and a 35% risk by age 70. They also have an increased risk for ovarian and pancreatic cancer with estimated lifetime risk 3 – 5% and 5 – 10%, respectively.

2. In the second case, ultrasound anomalies included aberrant right subclavicular artery, rocker bottom feet, fetal hydrops, and fetal ascites. Familial history for genetic disorders was negative. Fetal WES revealed a variant of unknown significance (VUS) mutation in the LMX1B gene. The LMX1B (LIM homeobox transcription factor 1-beta) gene is located on chromosome 9q33.3 which encodes a transcription factor that belongs to the LIM-homeodomain family of proteins.

Pathogenic variants in the LMX1B gene are causing focal segmental glomerulosclerosis 10 (MIM#256020) and Nail-patella syndrome (MIM#161200). Focal segmental glomerulosclerosis-10 (FSGS10) is an autosomal dominant kidney disease characterized by isolated glomerulopathy without extrarenal manifestations (PMID: 28059119). This renal disease is highly variable in severity and pathology, even within the same family. Nail-patella syndrome is also an autosomal dominant disease characterized by dysplastic nails, absent or hypoplastic patellae, elbow dysplasia, iliac horns, glaucoma and focal segmental glomerulosclerosis. Renal involvement is the major determinant of the prognosis for Nail-patella syndrome. Patients often present with varying degrees of proteinuria or hematuria, and can occasionally progress to chronic renal failure (PMID: 27450397).

When following up this case, the baby was born with no complications and presented no clinical symptoms, until present, at around 1 year old.

3. The third case presented with multiple abnormal findings on the second-trimester ultrasound exam including right aortic arch, severe hydronephrosis, caudal regression syndrome, and mesoaxial hand polydactyly. The fetal WES result shows a heterozygous pathogenic variant in HOXD13 gene c.820C>T(p.Arg274Ter). The c.820C>T (p.R274*) alteration, located in exon 2 (coding exon 2) of the HOXD13 gene, consists of a C to T substitution at nucleotide position 820. This changes the amino acid from an arginine (R) to a stop codon at amino acid position 274. Based on data from the gnomAD

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database, the HOXD13 c.820C>T alteration was observed in <0.01% of the total alleles studied.This couple decided for the termination of pregnancy.

4. In the fourth case, the WES referral was an abnormality of the cerebral structure – mega cisterna magna and ventriculomegaly.

Whole exome analysis for the detection of point mutation variants (nucleotide substitutions & deletion/insertion of some base pairs) associated with the clinical phenotype of the fetus (focus on 189 brain malformation gene panel) was performed and the result revealed a missense heterozygous variant in the ADNP gene with uncertain clinical significance (VUS).

The ADNP gene is located on chromosome 20q13.13 which encodes a homeodomain-containing zinc finger protein with transcription factor activity that is essential for brain formation. Pathogenic variants in the ADNP gene cause autosomal dominant Helsmoortel-van der Aa syndrome (MIM#615873), a neurodevelopmental disorder characterized by impaired intellectual development, motor delay, autism spectrum disorder, facial dysmorphisms, hypotonia, congenital heart disease, visual difficulties and gastrointestinal issues (PMID: 32758449). Morphological brain particularities include wide ventricles, cerebral atrophy, underdevelopment of the corpus callosum, delayed myelination, white matter lesions and cortical dysplasia (PMID: 29724491). The c.1612G>A (p.Glu538Lys) variant replaces glutamic acid with lysine at codon 538. The glutamic acid residue is strongly conserved and there is a moderate physicochemical difference between glutamic acid and lysine. The observed variant is absent in the gnomAD database.

In cases such as this, when the observed variant is a VUS variant, yet there are clinical characteristics to be observed in the patient as well as facial features, the variant should be very much taken into consideration and the case be thoroughly analyzed, as to corroborate with other studies or other cases reported by various researchers worldwide. The multidisciplinary team who managed this case advised for continuous follow-up of the pregnancy and the medical geneticists explained to the family the implications of the current finding.

5. In the fifth case, the ultrasound revealed the agenesis of the corpus callosum, limb malformation, intrauterine growth restriction. The prenatal WES testing identified the following variant: c.734A>C (p.Glu245Ala) in the TGFBR1 gene in a heterozygous status. This is a likely pathogenic (class 4) variant, de novo.

The TGFBR1 gene is located on chromosome 9q22.33 and encodes a transmembrane serine/threonine kinase receptor for transforming growth factor-beta. Monoallelic pathogenic variants in this gene have been associated with Loeys-Dietz syndrome 1 (Loeys-Dietz syndrome 1, MIM#609192) and are inherited by an autosomal dominant manner. Loeys-Dietz syndrome is characterized by hypertelorism, bifid uvula and/or cleft palate, and arterial tortuosity with widespread vascular aneurysm and dissection. Clinical features include microretrognathia, hypertelorism, exotropia, blue sclerae, craniosynostosis, malar hypoplasia, arachnodactyly, camptodactyly, talipes equinovarus, translucent skin, joint laxity, pectus deformity, dolichostenomelia. Loeys-Dietz syndrome patients have a high risk of aortic dissection or rupture at an early age and at aortic diameters that ordinarily are not predictive of these events (PMID: 19883511).

The c.734A>C (p.Glu245Ala) variant replaces glutamic acid with alanine at codon 245 and it is located in the kinase domain. The glutamic acid residue is highly conserved, and it shows large differences in physicochemical properties compared with alanine. According to the ACMG criteria (PM1, PM2, PM5, PP3), the c.734A>C (p.Glu245Ala) variant detected in the TGFBR1 gene is classified as likely pathogenic.

This case also yielded a pathogenic variant in HBB gene, inherited from the mother: c.-151C>T, responsible for the minor trait, Beta-thalassemia (AR).

The follow-up of this case shows that this baby was born, affirmatively without symptoms at the age of 1 year old. The Doppler echocardiography showed a slightly dilated abdominal Aorta, with no current hemodynamic importance.

6. In the sixth case, the couple were referred to genetic counseling due to being consanguineous and because the fetus showed Corpus Callosum agenesis and oligohydramnios at the fetal

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morphology. They are 2nd-degree cousins. At the time of the consultation, the gestational age of the pregnancy was 18 weeks.

The analysis through WES yielded a negative result, but with an incidental pathogenic variant in the BRCA2 gene, inherited from the mother. The variant was pathogenic (class 5): c.793+1G>A.

Genetic counseling was offered for the mother and all the relatives at risk.

7. This case was referred to our clinic by the attending obstetrician, at 19 weeks of gestation, for intracardiac echogenic focus, pyelectasis, long QT syndrome of the father, family history of sudden cardiac death, arrhythmias (without a genetic diagnosis).

The variant discovered was a VUS variant: KCNQ1:c.605-28A>G. Pathogenic monoallelic variants are responsible for: long QT syndrome 1, susceptibility to short QT syndrome 2, atrial fibrillation, familial, 3. After this result, we recommended Sanger sequencing for both parents to test the know mutation found in the fetus. The mutation was also present in the father, corresponded to his clinical phenotype, long QT syndrome 1, so we concluded that this variant was inherited from the father.

8. This case was referred for right aortic arch by our obstetrician colleague, at 17 weeks of gestation. After we performed SNP array and yielded a negative result, the team decided to undergo WES testing.

The prenatal WES yielded a negative result, the couple decided to continue with the pregnancy, the baby was born, affirmatively without symptoms at the age of 1.

9. For the ninth case, the fetus had sexual ambiguity on ultrasound morphology, intrauterine growth restriction, hypospadias and polyhydramnios. Because the pregnancy was already 20 weeks old, we decided to perform SNP array and WES at the same time.

The SNP array result was: arr[GRCh38]12p13.33p11.22(148769_30138756)x2 hmz,12q21.31q24.22(84757938_117685540)x2 hmz.

The prenatal WES was negative.

In the meantime, the couple decided to terminate the pregnancy, based on the ultrasound findings and the multidisciplinary team decided to perform constitutional karyotyping from the product of conception, keeping in mind the result of SNP array.

The constitutional karyotype of the fetus was: 47, XY, +12[7]/46,XY[23] (ISCN 2020), revealing a mosaic trisomy 12, in 23% cells, which could explain the fetal phenotype.

10. The last case was referred for genetic counseling and testing based on borderline bilateral ventriculomegaly, suspicion of hydrocephaly with Sylvian stenosis at fetal morphology. The SNP array was negative, so we continued with WES, which also yielded a negative result.

Even if the result for WES was negative in this case, we found two variants in accordance with the fetal phenotype, but without clinical impact on the diagnosis because the inheritance is autosomal recessive. The variants reported were LAMB1:c.3499C>T (p.Arg1167Ter) and PTPN23:c.2248C>A (p.Pro750Thr). Biallelic variants in LAMB1 gene are known to cause lissencephaly 5 and biallelic variants ain PTPN23 cause neurodevelopmental disorder and structural brain anomalies with or without seizures and spasticity.

The couple was offered genetic counseling and the whole multidisciplinary team had a very important role in managing this family.

We conclude that WES yielded a positive result for 50% of the cases where SNP- array was negative.

4. Discussion

Prenatal genomic diagnosis, genetic counseling, decisions based on informed consent require the intervention of a multidisciplinary team that includes an expert in maternal-fetal medicine with competence in prenatal ultrasound, geneticist, neonatologist, psychologist [14-17].

Prenatal diagnosis of a lethal genetic disease allows parents to make decisions related to the evolution of the pregnancy, including terminating the pregnancy under local legal conditions or preparing for the birth to take place in a neonatal intensive care center. In addition, it is possible to

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establish an adequate genetic counseling, to estimate a risk of recurrence and the available reproductive options [18-21].

Genetic counseling in prenatal WES

There is currently no available information related to the indications for the use of WES prenatally in the absence of fetal anomalies - so pre-test genetic consultation and counseling are essential.

Genetic counseling begins before the actual test for all cases, without exception, and is carried out by a medical genetic specialist. Aspects related to the benefits and limitations of the test, the parents' expectations, the necessity and usefulness of obtaining a definite diagnosis instead of uncertainty will be discussed. In addition, the pretest discussion must include information about the possible results (interpretation of variants) - the identification of variants that most likely explain the fetal phenotype, the identification of variants with uncertain clinical significance in genes that could be involved in the fetal phenotype or a negative result, without identifying any variant possibly linked to the phenotype. Variants with uncertain clinical significance will be able to be reinterpreted later, as new information becomes emerges online and becomes available in the literature [21-28].

Ethical aspects

The statement by de Jong and de Wert, starts a debate about how prenatal testing should be state insured, as to having the option to terminate a particular pregnancy where the future child will be born with severe disorders that might lead to the child's death or have the child be handicapped for life. State health services should remain impartial concerning couples' pre- and post-test choice whether the couple would like to keep or terminate the pregnancy, whilst offering the best support to uphold their wish. For example, if the couple would like to keep a pregnancy where the newborn would have either congenital affections or various other pathologies, the state services could book a consultation prenatally for a particular type of surgeon or organize a multidisciplinary team to better treat and tend to the needs of the patient. On the other side, should the couple choose to terminate the pregnancy, the state services could offer a variety of ob-gyn specialists who could provide them the best care before and after terminating the pregnancy [10,11].

A crucial step in genetic testing is the requirement of informed consent from the tested patients or the prenatally screened couple's pregnancy. The informed consent form should be comprised regarding pre- and post-test options, the lack of legal constraint, a specialized pathology management plan, assuring optimal perinatal care or in other cases, as far as palliative care, the limitations of the genetic testing and the limitations of the current worldwide knowledge.

5. Conclusions

In this article we present 10 cases which were tested through prenatal WES, showing the great diagnostic yield in comparison to SNP-array in our selected cases. As a closing remark, we wish to highlight the benefits of WES testing in prenatal cases. Further testing is needed to get a clearer picture of the prenatal diagnostic yield in the Romanian population. Genetic screening programs should be developed and implemented by the Department of Health together with healthcare professionals and a coordinating team should be assigned to evaluate and supervise these programs, to maintain and ensure their good use [29-32].

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