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Next-Generation Sequencing-Based Testing of Mendelian Disorders in Families Seeking Prenatal Diagnosis: An Analysis of 25 Cases

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Abstract

We retrospectively analysed the findings of 25 cases of next-generation sequencing-based prenatal diagnosis at our centre. Couples referred for prenatal genetic testing to prevent recurrence of genetic disorders in the family (diagnosed in a previous child or other family member), were included in the study. For couples who sought prenatal diagnosis in view of history of a previous child being affected with a suspected genetic condition such as intellectual disability, immunodeficiency, skin disorder like epidermolysis bullosa, etc., genetic workup of the proband was done first wherever the index case was available. Otherwise, carrier screening of the couple for recessive disorders was done. Next generation sequencing (NGS) -based clinical exome sequencing (CES) or whole-exome sequencing (WES) was used for index case workup or couple's carrier screening to identify the causative gene mutation(s). Once NGS identified causative variants in the proband or carriers, targeted mutation analysis was done in the prenatal sample. Sixteen percent of the couples were consanguineous. CES was the most common type of NGS testing applied (64%). The commonest indication for this testing was intellectual disability in a previous affected child of the couple (8/25). Of the other cases, antenatally detected anomalies (in a previous pregnancy) and genodermatoses accounted for 5 cases each. Others included one case each of progressive familial intrahepatic cholestasis (PFIC), Alagille syndrome, retinoblastoma, cystinosis, Leber congenital amaurosis, Waardenburg syndrome type 2E, and congenital neutropenia. In this study, we detected nineteen cases with autosomal recessive inheritance, five cases with autosomal dominant inheritance and one with X-linked recessive inheritance. Our study reiterates the fact that appropriate integration of NGS in the workup of families with Mendelian disorders has the potential to expand the existing armamentarium in prenatal diagnosis.

Keywords: Prenatal diagnosis, Next-generation sequencing, Whole-exome sequencing, Clinical exome sequencing

Introduction

Two to four out of hundred children are born with birth defects worldwide (March of Dimes, 2019). Birth defects/ developmental disabilities are caused due to chromosomal defects, single-gene disorders, teratogens, infections, and multifactorial causes. Diagnosis of monogenic disorders was challenging till the recent past, mainly due to the lack of easy genetic diagnostic tests and genetic heterogeneity associated with many disorders. Many cases with neurodevelopmental disabilities did not have clinical clues to probable causative gene / genes.

Disorders like epidermolysis bullosa have marked genetic heterogeneity, some genes being very large for Sanger sequencing. Hence, genetic testing by Sanger sequencing is very tedious and not practical or feasible most of the time. In recent years, NGS-based exome sequencing has rapidly evolved into a powerful tool for diagnosis of monogenic disorders and disease gene identification.

Many of the monogenic disorders that result in poor prognosis are both phenotypically and genetically heterogeneous and may not have structural anomalies and hence, cannot be diagnosed on antenatal ultrasound. Moreover, recurrence risk can be up to 25-50% depending upon the mode of inheritance of the disease. Requests for such prenatal diagnosis during pregnancy are not uncommon.

When a fetal anomaly is detected in the antenatal scan, prenatal testing that is offered currently includes quantitative fluorescence polymerase chain reaction (QF-PCR) or fluorescence in situ hybridization (FISH) for rapid diagnosis of aneuploidy, G-banded karyotyping, and chromosomal microarray analysis (CMA) for detection of copy number variations. The diagnostic yield of karyotype and QF-PCR/FISH is 5-35% depending upon the type of anomaly, and chromosomal microarray increases the detection rate to 6-10% beyond routine karyotype (Wapner et al., 2012). There are still many cases with fetal anomalies where an etiological diagnosis is not achieved as these techniques are not useful for monogenic disorders. With the advances in genomic technology and their increasing application in clinical practice, it is being realized that NGS-based testing can be very useful for genetic counseling and prenatal diagnosis. It can identify *de novo* as well as inherited gene mutations. The NGS-based analysis includes three major groups: clinical exome sequencing (CES), whole-exome sequencing (WES), and whole-genome sequencing (WGS). Exons are the protein-coding regions of the genome, which make up 1% to 2% of the total genome, but more than 85% of all disease-causing mutations are reported in these regions (Yang et al., 2014).

In clinical exome sequencing, only those genes are included, which are known to be disease causative, and the total number of genes in this panel would vary depending upon the laboratory. Generally, it can cover 5000- 7000 genes. In whole-exome sequencing, exons of all genes are tested, and testing would include around 20,000 genes. Whole-genome sequencing involves testing whole exons, introns and other parts of the genome, and is mainly used as a research tool currently.

The present retrospective study represents our experience with use of NGS-based testing for facilitating prenatal diagnosis in families with a previous child/other family member affected with a suspected Mendelian disorder.

Patients and Methods

A total of 25 couples who were referred for prenatal diagnosis, in view of a previous child or other family member having history suggestive of a genetic disorder, were included in the study. Couples with family history of intellectual disability, skin disorder, deafness, blindness, immunodeficiency etc., or ultrasound-detected fetal anomalies in a previous pregnancy, were selected for the study. The following three scenarios were encountered:

i] NGS testing done in the proband: This group consisted of a total of 18 cases (Cases 1-18), where a previous child was affected and the couple came for prenatal testing for the ongoing pregnancy. This group included cases of intellectual disability, epidermolysis bullosa, Walker-Warburg syndrome (**Figure 1a**), Meckel-Gruber syndrome (**Figures 1b, 1c**), progressive familial intrahepatic cholestasis (PFIC), Alagille syndrome, retinoblastoma, cystinosis, Leber congenital amaurosis, and Waardenburg syndrome type 2E.

A clinical geneticist did complete clinical evaluation of the proband and analysis of the clinical records, wherever available. Further, relevant investigations were done for the proband. Appropriate workup in cases with developmental delay included: tandem mass spectrometry (TMS), urine gas chromatography-mass spectrometry (GCMS), chromosomal microarray (750K), Fragile X screen, magnetic resonance imaging (MRI) of the brain, and neurophysiological studies (when indicated). Clinical exome/ whole-exome sequencing was advised as a second-tier test when results of the baseline workup and first-tier tests were normal, or as a first-tier test if the clinical presentation was strongly suggestive of a monogenic condition.

ii] NGS testing done in the couple: This group consisted of a total of five cases (Cases 19-23), where there was a strong suspicion of a genetic

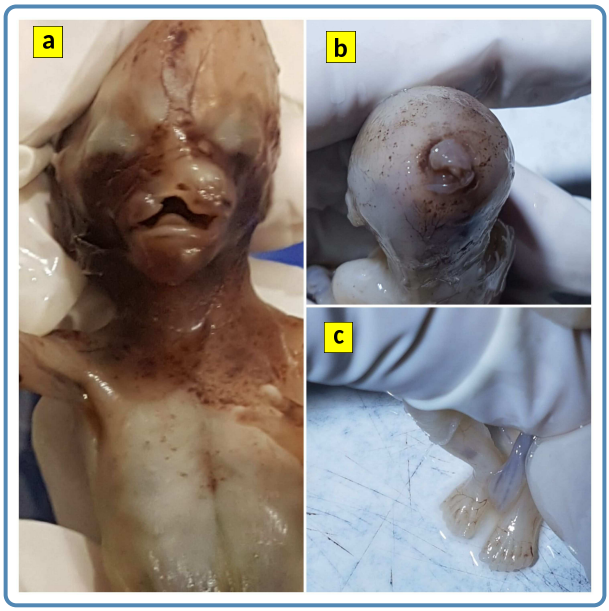

Figure 1 a) Autopsy findings of unilateral cleft lip and palate, in the fetus affected with Walker-Warburg syndrome (Case 9). b) & c) Autopsy findings of encephalocele and postaxial polydactyly in the fetus with Meckel-Gruber syndrome.

disorder based upon the history and investigations of the affected deceased child. This group included cases of homocystinuria, autosomal recessive polycystic kidney, ichthyosis, developmental delay, and congenital neutropenia.

iii] NGS done directly in the prenatal sample along with couple's NGS testing (trio analysis): This group had two cases (Cases 24-25). In one case, there was history of a previous child with hypohydrotic ectodermal dysplasia. In the other case, the ultrasound detected polycystic kidneys in the fetus, and the family history and pedigree were consistent with autosomal dominant polycystic kidney disease; the father and paternal grandmother of the fetus had features of polycystic kidneys and were under the care of a nephrologist.

Routine antenatal ultrasound/nuchal translucency scan and combined screening for common chromosomal aneuploidies were done in each of the pregnant ladies in the first trimester. Fetal samples were collected by either chorionic villus sampling (CVS) or amniocentesis after genetic counseling.

NGS-based testing was outsourced to genetic laboratories and the American College of Medical Genetics and Genomics/ Association for Molecular Pathology (ACMG/AMP) guidelines were used for reporting of variants (Richards et al., 2015).

Results

Out of the 25 couples who consulted us, most women belonged to the age group of 26-30 years (48%), followed by the age group of 31-35 years (28%). Ten couples consulted preconceptionally, while three families came in early pregnancy. Thus, 52% of cases came at the appropriate time when workup could be done.

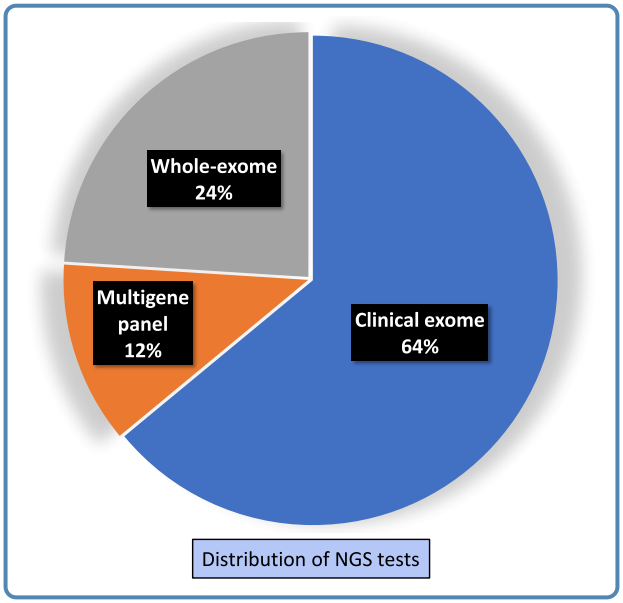

Figure 2 Distribution of types of next generation sequencing-based testing done in the study.

Four out of the twenty-five (16%) couples reported consanguinity. Sixteen out of the twenty-five cases underwent CES. Two couples with family history suggestive of polycystic kidneys and one couple with family history of childhood-onset blindness, underwent NGS-based multigene panel testing. WES was done in six cases (**Figure 2**). Fourteen out of 25 (56%) cases had prenatal testing by CVS, and six (24%) cases underwent amniocentesis as they presented in later gestation. In four (15%) cases, where ultrasound-detected fetal anomalies had been documented in a previous pregnancy, the couple declined invasive procedures due to fear of procedure-related risks, and elected for monitoring by ultrasound. In one case of Leber

congenital amaurosis, prenatal testing was not offered due to 100% risk of the disease to the fetus, as both partners had a homozygous mutation in the same gene (*LCA5*).

In this study, we detected 19 cases with autosomal recessive (AR) inheritance, 5 cases with autosomal dominant (AD) inheritance, and 1 with X-linked recessive (XLR) inheritance. Out of the 19 cases with AR disorders, both alleles (homozygous or compound heterozygous) had pathogenic variants in eleven, one variant was pathogenic and the other likely pathogenic in 3 cases, both alleles had likely pathogenic variants in 4 cases, and in 1 case both alleles had a variant of uncertain significance (VOUS). Of the variants identified in the 5 cases with autosomal dominant disorders, three were pathogenic variants and two were likely pathogenic variants. In one case with X-linked hypohidrotic ectodermal dysplasia, a pathogenic variant was identified.

Eight out of 25 (32%) families were referred with a previous child having developmental delay and seizures followed by five cases (20%) each with fetal anomalies detected in a previous pregnancy and skin disorders in a previous child. Clinical and molecular details of all cases are given in **Table 1**. The details of some illustrative cases from each of the three categories with the final molecular diagnosis are described hereunder:

• **Group I, Case 3: *NDUFS1* gene-associated mitochondrial complex I deficiency:** A 32-year-old lady presented for prenatal diagnosis. The family had lost dizygotic twin boys. One of them presented during the neonatal period with progressive encephalopathy and seizures after acute gastroenteritis. He also had high blood pressure, metabolic acidosis, and marginal elevation of lactate, and high plasma and cerebrospinal fluid lactate. The second twin also developed progressive lethargy and encephalopathy. MRI of the brain of both twins revealed similar results. It showed bilateral areas of diffusion restriction involving the white matter of centrum semiovale, periventricular white matter, cortical spinal tract, posterior limbs of internal capsules, middle cerebral peduncles, cervicomedullary junction, and posterior aspects of the cervical spinal cord. Areas of cavities with small foci of hemorrhage in bilateral semiovale and periventricular white matter were also seen. WES analysis of the twins revealed one heterozygous missense likely pathogenic variant in the *NDUFS1* gene namely c.1825A>G [p.Thr609Ala] in exon 16. Multiplex ligation-dependent probe amplification (MLPA) of the same gene was performed next, and this showed a duplication of exon 15- 17 on one allele. The missense variant was inherited from the father, and the duplication was inherited from the mother. The present pregnancy was evaluated for these two variants, and the fetus was found to be a carrier of the c.1825A>G variant.

• **Group I, Case 9: *POMT1* gene-associated Walker-Warburg syndrome:** Previous 2 pregnancies of this couple had been terminated. The first affected pregnancy was terminated for antenatally detected fetal encephalocele. In the second affected pregnancy, the mother was referred to us at 13 weeks of gestation and the ultrasound had revealed unilateral cleft lip and palate. A large cyst was identified in the posterior fossa measuring 8.4 mm in diameter, suspected as a precursor of Dandy-Walker malformation. Ventriculomegaly in the brain was observed with significant dilation of ventricular cavities. The chromosomal microarray on CVS was normal. After termination of the second affected pregnancy, fetal autopsy showed unilateral cleft lip and palate, small encephalocele and posterior fossa anomaly (**Figure 1a**). CES was done which showed a homozygous pathogenic frameshift variant c.1081C>T [p.Gln361Ter] in the *POMT1* gene. The results confirmed the genetic diagnosis of congenital muscular dystrophy-dystroglycanopathy with brain and eye anomalies type A1. It includes both the more severe Walker-Warburg syndrome (WWS) phenotype, and the slightly less severe muscle-eye-brain disease (MEB).

The option of prenatal diagnosis for future pregnancies was discussed with the couple. However, for the subsequent pregnancy, the couple underwent in vitro fertilization (IVF) with donor gametes.

• **Group I, Case 17: *LCA5* gene-associated Leber congenital amaurosis:** A primigravida consulted at 8-9 weeks of pregnancy as both she and her husband were diagnosed to have childhood-onset retinitis pigmentosa. NGS-based multigene panel testing for retinitis pigmentosa revealed the same homozygous pathogenic variant c.1151del [p.Pro384GlnfsTer18] in the *LCA5* gene in both of them, which confirmed the diagnosis of Leber congenital amaurosis in both. There was no history of consanguinity in their parents and also the couple was non-consanguineous. Prenatal testing was not offered in this case due to a 100% risk of biallelic mutations in the ongoing pregnancy.

Table 1 Clinical and molecular details of 25 cases included in the study [Group I- Cases 1-18, Group II- Cases 19-23, Group III- Cases 24 &25].

Case No.	Disease/ Gene In the family	Consan-guinity	Proband's sex and age/ Whether tested	NGS results of the proband	NGS/ Sanger sequencing of the couple	USG of the current pregnancy	Procedure done/ Result
1	Aicardi-Goutieres Syndrome/ *RNASEH2C*	No	1 year, Female/ Yes	Homozygous: NM_032193 c.205C>T [p.Arg69Trp] Pathogenic	NA	NT scan normal	CVS/ Fetus affected
2	Infantile systemic hyalinosis/ *ANTXR2*	Yes	5 months, Male/ Yes	Homozygous: NM_001145794 c.797-1G>A [Splice site variant] Pathogenic	Both partners heterozygous for variant	USG Normal	CVS/ Fetus carrier
3	Mitochondrial complex I deficiency/ *NDUFS1*	No	6 months, Male/ Yes	Compound heterozygous NM_005006 c.1825A>G [p.Thr609Ala] Likely Pathogenic & Duplication of exons 15-17 (MLPA) Likely Pathogenic	Partners heterozygous for one variant each	USG Normal	Amniocentesis/ Fetus carrier
4	Krabbe disease/ *GALC*	No	3 months, Male/ Yes	Compound heterozygous NM_001201402 c.967G>A [p.Gly323Arg] Pathogenic & c.328+1G>T [Splice site variant] Likely Pathogenic	Partners heterozygous for one variant each	USG Normal	CVS/ Fetus carrier
5	Biotinidase deficiency/ *BTD*	Yes	1 year, Female/ Yes	Homozygous NM_001370753 c.98_104delinsTCC [p.Cys33PhefsTer36] Pathogenic	Both partners heterozygous for variant	USG Normal	Amniocentesis/ Fetus carrier
6	Polymicrogyria/ *ADGRG1* or *GPR56*	No	2 years, Male/ Yes	Homozygous NM_001145774 c.739_745delCAGGACC [p.Gln247CysfsTer74] Pathogenic	Both partners heterozygous for variant	USG Normal	CVS/ Fetus carrier

7	Neuro-developmental disorder with or without hyperkinetic movements and seizures/ *GRIN1*	No	Male/ Yes	Heterozygous NM_001185090 c.2476C>T [p.Pro826Ser] Likely Pathogenic	Variant absent in both partners	USG Normal	Amniocentesis/ Fetus normal
8	Meckel-Gruber syndrome/ *TCTN2*	No	Fetus/ Yes	Compound heterozygous NM_024809.4 c.1895+1G>A (intron 6) Likely Pathogenic & Heterozygous large deletion in exon 5 Likely Pathogenic	-	USG Normal	Declined invasive testing
9	Congenital muscular dystrophy-dystroglycanopathy with brain and eye anomalies type A1/ *POMT1*	No	Fetus at 13-14 weeks/ Yes	Homozygous NM_001353193 c.1081C>T [p.Gln361Ter] Pathogenic	-	USG Normal	Underwent IVF with donor gametes
10	Epidermolysis bullosa/ *LAMC2*	No	1 year, Male/ Yes	Homozygous NM_018891 c.3374_3393del [p.Gln1125Hisfs] Pathogenic	Both partners heterozygous for variant	USG Normal	CVS/ Fetus carrier
11	Epidermolysis bullosa/ *COL7A1*	No	4 years Female, twins/ Yes	Homozygous NM_000094 c.25 G>C Likely Pathogenic	Both partners heterozygous for variant	USG Normal	CVS/ Fetus carrier
12	Laryngoonycho-cutaneous Syndrome and Epidermolysis Bullosa, Junctional, Herlitz Type/ *LAMA3*	No	1 year, Male/ Yes	Homozygous NM_198129 c.7056delG [p.Ile2353LeufsTer24] Pathogenic	Both partners heterozygous for variant	NT scan normal	CVS/ Fetus affected

13	Progressive familial intrahepatic cholestasis/ *ABCB11*	No	10 months, Female/ Yes	Compound heterozygous NM_021022 c.3695C>A [p.Ala1232Asp] Likely Pathogenic & c.3931delT [p.Tyr1311ThrfsTer3] Pathogenic	-	USG Normal	CVS/ Fetus affected
14	Alagille Syndrome/ *JAG1*	No	1 year, Male/ Yes	Heterozygous NM_000214 c.1899_1900delTG [p.Cys633Ter] Pathogenic	-	USG Normal	CVS/ Fetus not affected
15	Retinoblastoma/ *RB1*	No	8 years, Male/ Yes	Heterozygous NM_000321 c.115_124delCCCGGAGGAC Pathogenic	Variant absent in both partners	USG Normal	CVS/ Fetus normal
16	Cystinosis/ *CTNS*	No	8 months, Male/ Yes	Homozygous NM_001374495 c.759_781del [p. G258SfsTer30] Pathogenic	Both partners heterozygous for variant	USG Normal	CVS/ Fetus carrier
17	Leber Congenital Amaurosis 5/ *LCA5*	No	Couple, 28-30 years/ Yes	Couple homozygous NM_181714.3 c.1151del [p.Pro384GlnfsTer18] Pathogenic	-	USG Normal	-
18	Waardenburg syndrome type 2E/ *SOX10*	No	5 years, Female/ Yes	Heterozygous NM_006941.4 c.892del [p.Asp298ThrfsTer13] Pathogenic	Variant absent in both partners	USG Normal	Amniocentesis/ QFPCR-showed Trisomy 21
19	Combined methylmalonic aciduria and homocystinuria/ *MMAHC*	No	No	NA	Inconclusive in both partners	USG Normal	CVS/ Fetus carrier NM_015506.2 Heterozygous c.347T>C [p.Leu116Pro] variant
20	Autosomal recessive severe congenital neutropenia/ *JAGN1*	Yes	No	NA	Both partners heterozygous NM_032492.4 c.17G>A [p.Gly6Asp] Likely Pathogenic	USG Normal	CVS/ Fetus Carrier

21	Ichthyosis, developmental delay, seizures/ *ELOVL4*	Yes	No	NA	Both partners heterozygous NM_022726.3 c.257T>A [p.Met86Lys] Likely Pathogenic	NT scan normal	CVS/ Fetus affected
22	Autosomal recessive polycystic kidney/ *PKHD1*	No	Yes	NA	Both partners heterozygous NM_001009944 c.1491delG [p.Gln498ArgfsTer9] Pathogenic	USG Normal	Declined prenatal testing as ultrasound was normal
23	Autosomal recessive polycystic kidney/ *PKHD1*	No	Yes	NA	Partners heterozygous for 1 variant each NM_001009944 Husband: c.403C>T, [p.Gln135Ter] Pathogenic Wife c.5199_5201del, VOUS	USG Normal	Declined prenatal testing as ultrasound was normal
24	Autosomal dominant Polycystic Kidney/ *PKD1*	No	Yes	Heterozygous NM_001009944 c.10552G>T [p.Glu3518Ter] Pathogenic	Affected husband heterozygous for variant	USG revealed bilateral enlarged kidneys with mildly increased parenchymal echogenicity	Amniocentesis/ Fetus affected
25	Hypohidrotic ectodermal dysplasia-1/ *EDA*	No	No	NA	Wife-Heterozygous NM_001005613.4 c.1045G>A [p.Ala349Thr] Likely Pathogenic	USG Normal	Amniocentesis/ Fetus affected

NGS: Next-generation sequencing; USG: Ultrasonogram; CVS: Chorionic villus sampling; NA: Not available

• **Group 1, Case 18: *SOX10* gene-associated Waardenburg syndrome type 2E:** A 31-year-old second gravida lady (G2P1+0) came to us in early pregnancy. The couple was non-consanguineous, and their five-year-old daughter had motor delay, deafness, heterochromia of iris, and skin hypopigmentation. Deafness was noted at ten months of age, and she had undergone a cochlear implant at 1.5 years of age. She also had history of constipation since 6 months of age. Trio WES was done in the proband and the couple. It identified a heterozygous single base-pair deletion in exon 4 of the *SOX10* gene, resulting in a frameshift and premature truncation of the protein. This mutation was not present in the parents. The risk of recurrence was small, but we offered amniocentesis at 16 weeks gestation. Antenatal ultrasound showed no markers of aneuploidy. However, QF-PCR which was done to exclude aneuploidy, revealed trisomy 21. The couple opted for termination of the pregnancy and further processing of the prenatal sample was not done.

• **Group II, Case 20: *JAGN1* gene-associated congenital neutropenia:** A consanguineously married couple were referred in early pregnancy at 6-8 weeks gestation with history of death of two previous male children due to recurrent infections, including respiratory infections and acute gastroenteritis, within 30 days of life. Investigations in the affected children had revealed severe neutropenia, leukopenia with reduced CD3, CD4, CD19, and CD56 cell counts and reduced IgM and IgA. The bone marrow biopsy of one child was suggestive of childhood myelodysplastic syndrome or refractory cytopenia; the child was clinically suspected to be affected with immunodeficiency or bone marrow myelodysplasia. The couple's NGS testing showed that both were heterozygous carriers for the likely pathogenic variant c.17G>A [p.(Gly6Asp)], in the *JAGN1* gene. CVS was performed at 12 weeks of pregnancy and the fetus was found to be heterozygous for this variant.

• **Group II, Case 21: *ELOVL4* gene-associated developmental delay and ichthyosis:** This consanguineous couple was referred prior to their second pregnancy, for genetic counseling and evaluation, as they had lost their first daughter at 14 months of age. The child had global developmental delay and seizures. She also had ichthyosis noticed since birth. As the proband was not available for testing, we did carrier screening by NGS. A heterozygous missense variant c.257T>A [p.(Met86Lys)] in the *ELOVL4* gene was identified in both the husband and wife. Though this variant was classified as a VOUS, the clinical history of the affected deceased child was consistent with the features of *ELOVL4*-associated ichthyosis-spastic quadriplegia-mental retardation syndrome. Therefore, this variant was considered to be the disease-causing variant and we offered prenatal testing by CVS at 12 weeks after explaining the limitations of prenatal testing based on VOUS. The fetus was found to be homozygous for the *ELOVL4* gene variant.

Discussion

In this case series, we discuss the findings of NGS-based testing in families referred for prenatal diagnosis in view of history of a suspected genetic disorder in the family. In 18 cases out of the total 25, the proband was available for testing (Group I). Out of these 18, work up was done in the preconception period in fifteen families and in early pregnancy in 2 cases; only the case with biotinidase deficiency came in the second trimester. Couple carrier testing by NGS was performed in 5 cases where the proband was no longer alive and where the proband's evaluation had not been completed (Group II). Two cases underwent direct fetal sampling as they presented to us in the second trimester.

Evaluation of the proband during the preconception period is the best way of approaching genetic counseling and planning for prenatal diagnosis. Referral during early pregnancy also allows the option of prenatal testing. Late referral is associated with limitations in providing prenatal diagnosis due to time constraints. Sometimes prenatal sampling may be done simultaneously with testing of the proband or the couple. The limitations in such a situation need to be documented as in spite of collecting the fetal sample through invasive methods, accurate prenatal diagnosis may not be possible.

Prenatal genetic testing should be offered when pathogenic or likely pathogenic variant(s) consistent with the clinical history is/ are detected. VOUS present counseling challenges to the clinician and dilemmas to the family for decision-making.

In group III, trio testing (in prenatal sample and the couple simultaneously) was done. Trio analysis has higher diagnostic yields compared with non-trio analysis. It allows for the identification of *de novo* variants, determination of phase for biallelic variants, and confirmation of carrier status

in both parents when a homozygous variant is detected. (Monaghan et al., 2020).

Pretest counseling is ideally provided by a professional trained in genetics. In this study, the biggest challenge we faced was of clinical correlation of the NGS-based analysis. Most of the cases referred to us were in the preconception period or early pregnancy, so the prenatal diagnosis was done at 12 weeks and analysis was completed within the 20 weeks limits of termination. In six cases where we performed amniocentesis, four showed normal results and so termination of pregnancy was not needed. So, early referral is the key factor in the management but women should not be denied prenatal diagnosis if they present later in pregnancy as some of them would abort without testing and this may be a normal baby! This is because risk of recurrence is 25% in autosomal recessive disorders. With the use of prenatal exome sequencing, the turn-around time has to be rapid to maintain all aspects of reproductive choice.

One-fifth of our referrals were related to ultrasound-detected anomalies. These included two cases of polycystic kidneys detected as large bright echogenic kidneys on the ultrasound. As the index case was not available, we utilized NGS-based carrier analysis. Exome sequencing can identify genetic etiologies in cases with fetal anomalies, particularly recurrent cases or when there is a history of consanguinity or when clinical features are suggestive of a monogenic syndrome e.g., Meckel-Gruber syndrome.

Two recent large cohort studies have studied the diagnostic yield of exome sequencing in fetuses with one or more ultrasound anomalies and normal karyotype and microarray. The PAGE study included 610 fetuses with ultrasound anomalies, from the United Kingdom. They reported a pathogenic variant in 8.5%, and VUS in 3.9% of cases that was considered possibly pathogenic (Lord et al., 2019). Petrovski et al. reported on 234 fetuses with ultrasound anomalies and normal results on karyotype and CMA. They reported a pathogenic variant in 10% of such fetuses. Detection rate was 6% in fetuses with a single anomaly, whereas 19% of fetuses with more than one anomaly had a pathogenic genetic variant (Petrovski et al., 2019). These studies suggest that prenatal exome sequencing may provide clinically relevant information for the management of fetal anomalies identified in pregnancy and this will guide genetic counseling and prenatal diagnosis in future pregnancies.

Conclusion

The application of NGS-based testing has the potential to facilitate prenatal testing, and this can be employed in various scenarios as illustrated by our experience. The importance of diagnosis of the proband with a possible monogenic disorder preconceptionally needs to be stressed. Equally important is genetic testing of stillbirths or neonatal deaths, or at least storing a piece of umbilical cord or blood sample in an EDTA tube for further testing. The powerful genomic technique of NGS needs to be appropriately used for the benefit of families with poor reproductive outcomes.

Acknowledgments: Parag Agarwal and Anisha Raju, THB, Gurugram for assisting in the final formatting of the manuscript.

Funding: No funding received

Conflict of interests: None

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