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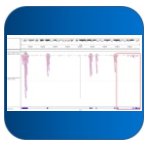

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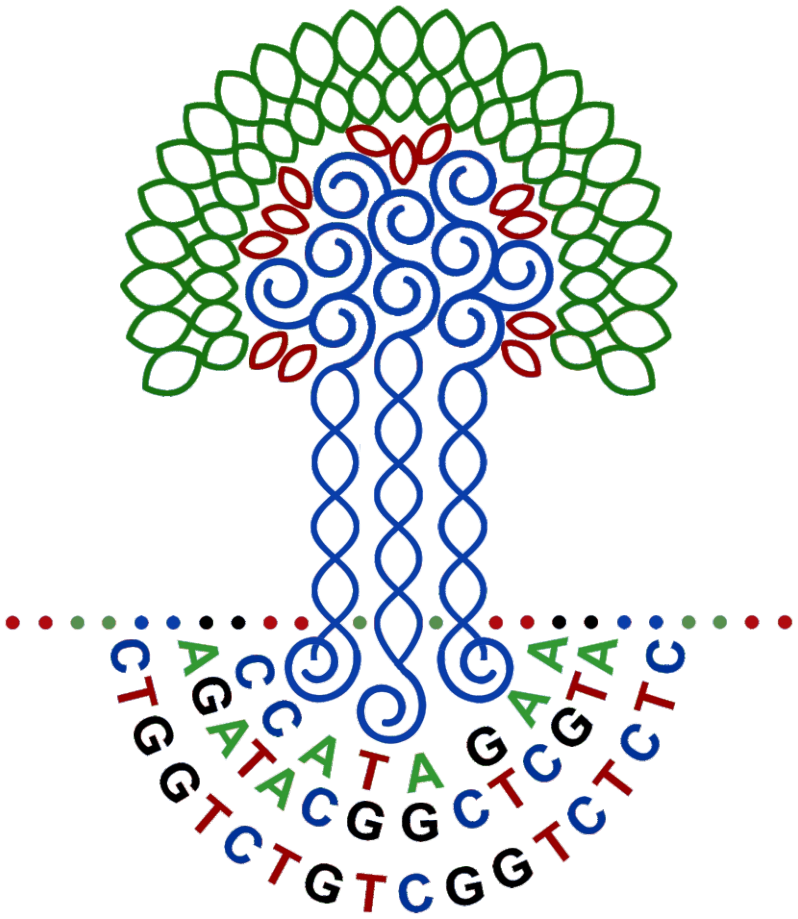



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# PhotoQuiz - 54

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This 4-year-old female child was referred for evaluation of short stature, waddling gait, and bowing of legs. Her serum biochemical parameters (normal values given in the brackets) were as follows: parathyroid hormone: 1.81pmol/l (1.1-6.6); 25 hydroxy vitamin D3: 79.06 nmol/l (50-250); calcium: 9.7mg/dL (8.8-10.8); phosphorus: 5.6mg/dL (3.2-5.8); and alkaline phosphatase: 331 IU/L (100-320). Identify the condition.

Please send your responses to editor@iamg.in
Or go to http://iamg.in/genetic_clinics/photoquiz_answers.php
to submit your answer.

## Answer to PhotoQuiz 53

Neu-Laxova syndrome (OMIM #256520 & #616038)

Neu-Laxova syndrome (NLS) is a rare autosomal recessive disorder characterized by microcephaly, typical facial dysmorphism in the form of proptosis, hypertelorism, micrognathia, ear anomalies, flattened nose and a round gaping mouth, a short neck, cleft lip/ palate, ichthyosis, intrauterine growth restriction, intracranial anomalies (including lissencephaly, cerebellar hypoplasia, and agenesis of the corpus callosum), limb deformities, cardiac and renal anomalies, edema, and hypoplastic lungs. Majority of affected babies die in the perinatal period. It is caused by biallelic variants in the *PHGDH* gene (*606879; NLS1) or the *PSAT1* gene (*610936; NLS2).

**Correct responses were given by:**

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GeNeDit

# Think of Rare Commonly!

## Editorial

Monogenic diseases are rare! But presentations of many of them overlap with, or are similar to those of common non-genetic or multifactorial disorders. Studies on novel genes of autoinflammatory disorders described in the GenExpress in this issue highlight the same point. These papers talk about gain-of-function mutations in genes involved in innate immunity. The presenting features of these monogenic autoinflammatory disorders are fever and '–itis', associated with haematological markers of inflammation; the features are similar to those of infections or autoimmune disorders. Recurrent episodes in the absence of immunodeficiency make one suspect that these can be autoimmune disorders. More than 100 autoimmune disorders are delineated, many of them with overlapping features. Development of the speciality of clinical immunology and expert management by the specialists has improved the outcomes of autoimmune disorders. But rarely the auto-antibodies are conspicuous by their absence in clinically suspected autoimmune disorders and in such scenarios, testing of multiple genes in one go clinches the diagnosis of monogenic auto-inflammatory disorders.

Autoinflammatory and autoimmune disorders are inflammatory disorders due to defects in the immune system. The autoinflammatory disorders are due to defects in the genes involved in innate immunity and its pathways. The inflammation starts by itself without known external triggers like microorganisms. On the other hand, autoimmune disorders are due to abnormalities of adaptive immunity where the body mistakes a cell type that is actually "self" as "other". These are akin to 'learning mistakes', while in autoinflammatory disorders the 'mistakes are in the basic knowledge' of the immune competent cells. As mentioned previously, the resulting symptoms are very similar. Presence of family history or consanguinity can help in clinical suspicion in addition to documented absence of autoantibodies. Some autoimmune disorders also have family history of other autoimmune disorders as they also have multifactorial etiology which includes a genetic component. Some autoinflammatory disorders manifest during fetal life or infancy and have overlapping features with infective disorders. This group includes pseudo-TORCH syndrome and Aicardi-Goutieres syndrome for which 3 and 9 causative genes are known till date. Correct diagnosis is important not only for appropriate management but also for genetic counseling as there may be 25 or 50% risk of recurrence in the family members. For severe disorders with early lethality and neurological involvement, the option of prenatal diagnosis also may be useful for the families. Specific targeted therapies also may become available as the genetic diagnosis provides knowledge of the molecular pathways involved in disease pathogenesis.

The monogenic autoinflammatory disorders should open our minds to rare monogenic causes of common presentations and stress the need for considering these etiologies in common presentations. Similar overlap is seen in other diseases with heterogeneous etiologies like diabetes mellitus, nephrotic syndrome, cardiomyopathy, hypertension, cataract, etc. In fact, there are monogenic causes for each of these clinical conditions, even though more often they are associated with multifactorial etiology. Understanding the genetics of rare monogenic disorders also paves the way for obtaining insights into the pathogenesis of common multifactorial disorders, which in turn helps in identifying therapeutic targets for novel drug development. It is thought that the genetic variants in the genes for monogenic disorders may be contributing as susceptibility loci for common multifactorial disorders.

Don't think of rare diseases rarely if you don't want to miss any diagnosis!

Dr. Shubha Phadke
1st October, 2021

Clinical Vignette

# Limitations of Whole-Exome Sequencing: Report of a Child with X-Linked Adrenoleukodystrophy

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## Abstract

X-linked adrenoleukodystrophy is the most common disorder of peroxisomal beta-oxidation of very long chain fatty acids. It presents with three distinct phenotypes, including the childhood cerebral form, adrenomyeloneuropathy and Addison's disease. It is an X-linked disorder caused by pathogenic variants in the *ABCD1* gene. Our case describes the diagnostic odyssey in a six-year-old child with neuroregression in the form of progressive loss of hearing and vision, and neuroimaging features highly suggestive of adrenoleukodystrophy. This brings out the inadequacies of the apparently impregnable whole exome sequencing, due to partial coverage of the *ABCD1* gene. We also enunciate the importance of clinical acumen and the need for collaboration with experts in the field in order to reach a quicker and more accurate diagnosis, especially in treatable disorders like these.

***Keywords:*** *ABCD1*, X-linked adrenoleukodystrophy

## Introduction

X-linked adrenoleukodystrophy (ALD) is the most common disorder of peroxisomal beta oxidation with an estimated prevalence of 1 in 17000 (Bezman et al., 1998; Kok et al., 1995; Raymond et al., 1999). The disorder presents as three distinct phenotypes: the childhood-onset cerebral form, adrenomyeloneuropathy and adult-onset Addison's disease. Neuroimaging shows a characteristic pattern of symmetric enhanced $T_2$ signal in the parieto-occipital region with contrast enhancement (Heubner et al., 1897; Raymond et al., 1999). We present the diagnostic dilemma encountered in a six-year-old boy with adrenoleukodystrophy, highlighting the various fallacies in testing and the crucial role of clinical acumen in such situations.

## Clinical report

A 6-year-old boy, fourth in birth order, presented to the genetic clinic with the chief complaints of progressive loss of ambulation and impairment of vision and hearing. Parents were married non-consanguineously. Antenatal, perinatal and neonatal periods were uneventful. The development was age-appropriate until the age of 4 years 9 months, when he started falling and bumping into objects and needed aid for ambulation. A simultaneous loss of hearing was also observed. Both hearing and vision loss were progressive and at presentation, he was unable to hear or see at all. On examination, there was no facial dysmorphism. Anthropometry was within normal limits. He was lean and average built. He was irritable and non-responsive to verbal commands. Central nervous system examination revealed spasticity in bilateral lower limbs with brisk deep tendon reflexes. Upper limb tone and reflexes were normal. Rest of the systemic examination was non-contributory. Magnetic resonance imaging (MRI) of the brain revealed characteristic T2 hyperintensities in the parieto-occipital lobe with contrast enhancement in the diffusion-weighted images (DWI), highly suggestive of adrenoleukodystrophy. Plasma lactate, ammonia and glucose were normal. Plasma very long chain fatty acids (VLCFA) analysis did not reveal any abnormalities.

Clinical diagnosis of ALD was suspected but as the VLCFA analysis was normal (repeated twice on same sample), whole exome sequencing (WES) was ordered instead of Sanger sequencing. It revealed a homozygous missense variant in the *EPRS1* gene, c.223C>T(p.His75Tyr). The clinical phenotype seemed to be consistent with that of our patient and the variant was segregated in the parents, but this remained a variant of uncertain significance according to revised American College of Genetics and Genomics

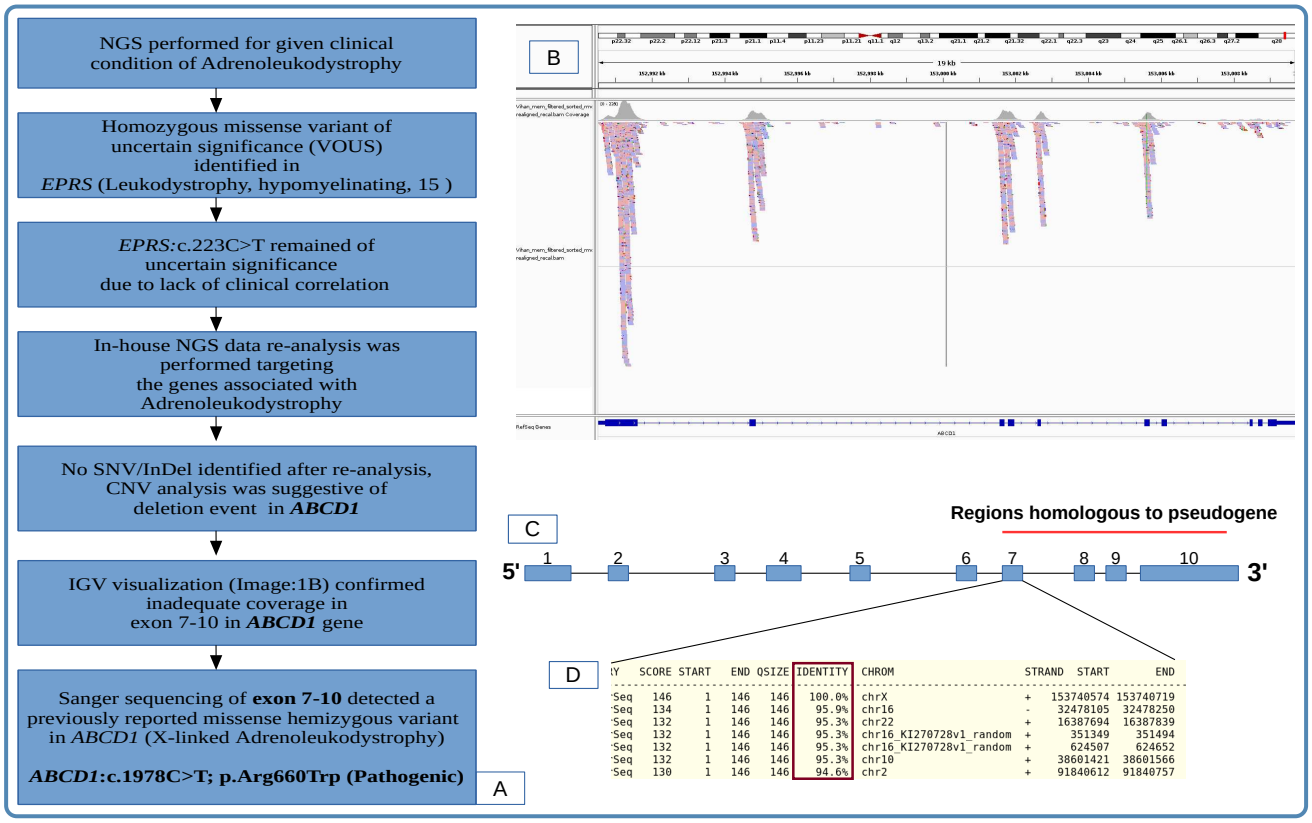


**Figure 1** A) Flowchart showing the steps which led to confirmation of the diagnosis of X-linked adrenoleukodystrophy in the child. B) Integrated Genome Viewer (IGV) visualization of NGS reads mapped to ABCD1 gene. Rectangular box (red colored) indicates inadequate coverage in the exons 7-10 region. C) Diagrammatic representation of the ten (10) exons in *ABCD1* gene. Eichler et al. (1997) identified that a 9.7-kb segment encompassing exon 7-10 of *ABCD1* was duplicated to 2p11, 10p11, 16p11, and 22q11. Due to high homology between the pseudogene and the *ABCD1* exon 7-10 region, care should be taken while analyzing the *ABCD1* gene. D) Pairwise alignment of exon 7 in *ABCD1* indicating sequence homology with chr16, chr22, chr10, chr2. Results are arranged in decreasing order based on the score generated by BLAT (box).

(ACMG) criteria (Richard et al., 2015). Therefore, the diagnosis of *EPRS*-related hypomyelinating leukodystrophy was not confirmed.

Since the phenotypic features were suggestive of ALD, opinion of world experts was sought for this patient and a repeat biochemical testing was done. C26:0-lysophosphatidyl choline (C26:0-lysoPC) was performed on the dried blood spot and was elevated (653 nmol/L, ref: 29-72). This brought back the focus to ALD. WES data re-analysis detected no pathogenic variant in the *ABCD1* gene. Further, it was noted that the exons 7-10 of *ABCD1* were not covered adequately as compared to other exons of the same gene (**Figure 1B**). Hence, the possibility of a deletion at exon level could not be ruled out by looking at the data pattern. To rule out any possibility of exon level deletion, multiplex ligation-dependent probe amplification (MLPA) was performed and this was normal. Finally, *ABCD1* gene Sanger sequencing was performed in view of incomplete coverage of certain exons. A hemizygous missense, previously reported variant was identified, in exon 9 of *ABCD1*, ENST00000218104.31: c.1978C>T (p. Arg660Trp). This variant was described by Kok et al. in 1995 in an Italian proband and was classified as likely pathogenic by the revised ACMG criteria (Kok et al., 1995). The flowchart depicting the sequence of testing leading to final diagnosis is shown in **Figure 1A**.

This case describes the diagnostic odyssey in a child with ALD due to initial misleading exome results. Next-generation sequencing has brought a paradigm shift in the diagnosis of

genetic disorders. WES has greatly increased the diagnostic yield of genetic disorders, especially those with a non-specific phenotype.

Nevertheless, like every new technology, it has its own promises and pitfalls. It has been recently shown that even current WES platforms have problems in sufficiently capturing the whole exome (Meienberg et al., 2015). One of the reasons for false negative reports of WES is the presence of pseudogenes which are non-functional segments of DNA that resemble functional genes. Most arise as superfluous copies of functional genes, either directly by DNA duplication or indirectly by reverse transcription of an mRNA transcript. Due to the great degree of sequence homology with functional genes, exome sequencing data is often inaccurate in genes which have associated pseudogenes.

WES for this patient was performed as the biochemical testing was unyielding. But exome data was skewed and missed the variant due to the presence of a pseudogene. The exons 7 to 10 of the *ABCD1* gene share sequence homology with similar sequences present on chromosomes 2, 10 and 16 and 22 (pseudogenes). This resulted in alignment mismatch for some reads (**Figures 1C, 1D**). Thus, it should be borne in mind that in presence of a high clinical suspicion, targeted testing by Sanger sequencing of a gene may be a preferable modality of testing.

Although VLCFA analysis is highly sensitive and specific for X-linked ALD, pathognomonic increased plasma VLCFA values are not always found in X-ALD. Both false positive due to hemolysis of the sample, non-fasting sample, or a ketogenic diet and a false negative, due to consumption of products like rapeseed oil or mustard seed oil that are rich in erucic acid (C22:1) can lead to the lowering of C26:0 (Engelen at al., 2012). Further, now there is availability of alternate biomarkers like C26:0-lysoPC which is performed on dried blood spots and has a much higher discriminative power than the plasma VLCFA analysis (Wanders et al., 2018).

In conclusion, this case throws light on the importance of a systematic step-wise approach in establishing the correct molecular diagnosis. Elevated VLCFA levels may not always be conclusive for ALD and alternative biochemical markers such as C26:0-lysoPC should be considered. Further, WES is not the absolute end-all of genetic testing and it is worthwhile to choose the molecular test with maximum coverage for the target gene/genes. Another learning point from this patient is to not label a diagnosis on the basis of variants of uncertain significance, in the absence of a highly specific phenotype. Lastly, we emphasise on the need for collaboration and discussion of patients with experts in order to provide them with the quickest and most accurate diagnosis.

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GeNeViSTA

# 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.

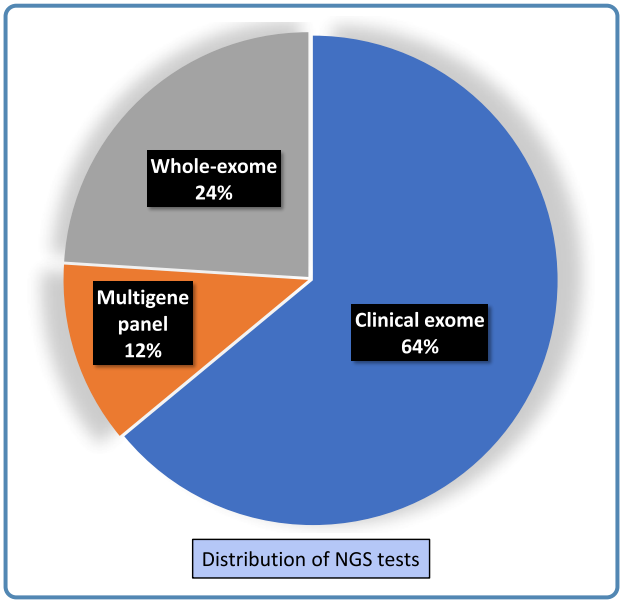


**Figure 2** Distribution of types of next generation sequencing-based testing done in the study.

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.

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GeNeViSTA

# Gaucher Disease: A Case Series

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## Abstract

Gaucher disease (GD) is the commonest lysosomal storage disorder (LSD). We report a case series of GD from a single centre of North India. Over a 2-year period, 21 children referred with chronic splenohepatomegaly, after ruling out hemolysis and portal hypertension, were screened for GD. GD was diagnosed in 13 of them. Initial screening was done on a dried blood spot sample. Nine of them underwent molecular genetic testing and all 9 had the homozygous mutation c.1448 T>C; p.Leu483Pro (previously named as p.Leu444Pro).

***Keywords:*** Gaucher disease, splenohepatomegaly, *GBA* mutation.

## Introduction

Gaucher disease (GD) is the commonest lysosomal storage disorder (LSD) with an estimated global incidence of 1: 40,000 to 1: 60,000 live births (Mistry et al., 2007). Globally, type I GD accounts for around 95% of cases. Interestingly, type III GD is relatively more common in India as compared to the western population (Puri et al., 2018). We report a case series of GD from a single center of North India.

## Patients and Methods

Children less than 16 years of age, with predominant complaints of splenohepatomegaly of more than 6 months duration and no evidence of hemolytic anemia and portal hypertension, were prospectively evaluated from June 2017 to June 2019. Hemolytic screening included complete blood count, reticulocyte count, peripheral blood smear examination and lactate dehydrogenase assay. Portal hypertension screening included a Doppler ultrasound of the abdomen. Children having any abnormality on these tests were evaluated further accordingly.

Patients with no evidence of hemolytic anemia or portal hypertension were initially screened for GD by measuring beta-glucosidase enzyme activity using the dried blood spot (DBS) kit, under the "Disha" diagnostic support service. All children were also referred for a detailed ophthalmology examination for cherry red spot and abnormal eye movements. Skeletal survey was not performed. Of those screened positive for GD on DBS, mutation analysis and plasma chitotriosidase assay were done in 9 and 10 children, respectively. Children having normal beta-glucosidase levels were advised to undergo sphingomyelinase enzyme assay in peripheral blood leucocytes (for Niemann-Pick disease type A/B), followed by clinical exome sequencing test, if the enzyme levels were normal.

## Results

Twenty-one children were evaluated during the study period. GD was diagnosed in 13 children and Niemann-Pick disease type B was diagnosed in 3 (**Figure 1**). The remaining 5 children were advised further testing but could not get it done and were lost to follow up.

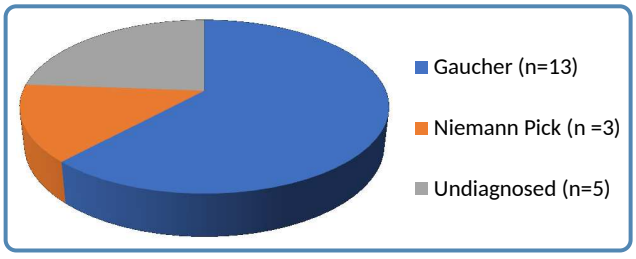


**Figure 1** Etiology in patients with splenohepatomegaly of more than 6 months duration (n=21).

**Table 1** Characteristics of patients with Gaucher disease in the study cohort.

| Sl. No. | Age at diagnosis (years) | Sex | Liver (cm palpable) | Spleen (cm palpable) | Hb gm % | Total leucocyte count/ cu mm | Platelet count/ cu mm | Mutation in *GBA* gene | Plasma chitotriosidase (nmol/ml/hr) | Beta-glucosidase (normal reference range) | ERT sessions during and after study period | Response to ERT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 8 | F | 6 | 23 | 8.5 | 3200 | 14000 | NA | NA | 1.1 micromol/L of blood/hr (2.3-16) | None | NAP |
| 2 | 2 | M | 0 | 17 | 7.3 | 2300 | 100000 | c.1448T>C; p.L483P | 59922 | 0.608 nmol/hr/mg protein (4-32) | 6 + 11 | CBC: Hb/TLC/ Plt: 9/2.8/1.2; Spleen 12 cm palpable |
| 3 | 1.5 | F | 2 | 8 | NA | NA | NA | NA | NA | 0.8 micromol/L of blood/hr (2.3-16) | None | NAP |
| 4 | 1.5 | F | 5 | 5 | 5.6 | 3000 | 90000 | NA | NA | 0.9 micromol/L of blood/hr (2.3-16) | None | NAP |
| 5 | 3 | M | 9 | 0 | 8.3 | 4000 | 170000 | c.1448T>C; p.L483P | 1041 | 1.9 micromol/L of blood/hr (2.3-16) | 0 + 15 | NAP |
| 6 | 1.1 | M | 2 | 8 | 10.6 | 5500 | 230000 | c.1448T>C; p.L483P | 750 | 0.75 micromol/L of blood/hr (2.3-16) | None | NAP |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 7 | 2 | M | 5 | 0 | 9.5 | 5000 | 170000 | c.1448T>C; p.L483P | 1361 | 1.58 micromol/L of blood/hr (2.3-16) | None | NAP |
| 8 | 2.5 | M | 0 | 11 | 9.4 | 2750 | 125000 | c.1448T>C; p.L483P | 1178 | 1 micromol/L of blood/hr (2.3-16) | None | NAP |
| 9 | 1.5 | M | 2 | 12 | 8 | 2600 | 90000 | c.1448T>C; p.L483P | 2.79 | 1.8 micromol/L of blood/hr (2.3-16) | None | CBC: Hb/TLC/Plt: 9.5/2.9/1.1; Spleen 8 cm |
| 10 | 3.5 | M | 2 | 10 | 8.3 | 7000 | 190000 | c.1448T>C; p.L483P | 1350 | 0.74 micromol/L of blood/hr (2.3-16) | 0 + 12 | NA; ERT & FU with nearby pediatrician |
| 11 | 1.5 | F | 2 | 7 | 9.3 | 7500 | 210000 | c.1448T>C; p.L483P | 600 | 0.94 micromol/L of blood/hr (2.3-16) | 0 + 12 | NA; ERT & FU with nearby pediatrician |
| 12 | 1.5 | M | 2 | 8 | NA | NA | NA | NA | 12947.3 | 0.43 nmol/hr/mg protein (4-32) | None | NAP |
| 13 | 2 | M | 8 | 12 | 9 | 3750 | 115000 | c.1448T>C; p.L483P | 1232 | 1.38 micromol/L of blood/hr (2.3-16) | None | NAP |

NA - Not available; NAP - Not applicable; M - Male; F - Female; CBC – Complete blood counts; Hb- Hemoglobin; TLC – Total leucocyte count; Plt – Platelet count; ERT – Enzyme replacement therapy; FU – Follow-up

The features of the GD patients are shown in **Table 1**. Eleven of the 13 children belonged to the Muslim community and history of parental consanguinity was present in 4 of them. There were 2 pair of siblings with GD (patient nos. 4 & 5, and 10 & 11) in this series. Apart from these, two children had a positive family history of sib death (patient nos. 1 & 2) with GD. The most common presenting symptom in our series was abdominal distension. The age of presentation ranged from 6 to 36 months and the median was 12 months. The age at diagnosis ranged from 13 months to 96 months with a median of 24 months.

Four patients had history of receiving blood transfusion and two of them had undergone splenectomy before referral. None had history of any bleeding manifestations or any significant bone pain or fracture.

Eight patients of GD hailed from a single district i.e., the Churu district of Rajasthan (**Figure 2**). Ophthalmological evaluation records were available in 10 children and none of them had cherry red spot or any abnormal eye movements. Hence all our patients were categorized as GD type I. Three children (patient nos. 1, 4 and 9) succumbed to the disease, due to inability to get enzyme replacement therapy (ERT).

There was anemia in all (100 %) with a mean hemoglobin of 8.65 gm/dl and platelets were low (< 1.5 lac/µL) in 4 (30%).

The beta-glucosidase enzyme level in the dried blood spot sample was low in all patients (mean level in patients 1.07 nmol/hr/mL; normal reference range of 2.3-18.4 nmol/hr/mL) suggesting the diagnosis. Out of the 10 patients in whom plasma chitotriosidase assay was done, nine had raised levels and one (patient no. 9) had a normal value, with a mean value of 8931 nmol/ml/hr (range in patients 600 – 59922 nmol/ml/hr; normal reference range of 0-90 nmol/ml/hr).

All nine patients had the same homozygous mutation c.1448 T>C; p.Leu483Pro (previously named as p.Leu444Pro). Though the phenotype of our patients is suggestive of type 1 GD, the genotype i.e., p.Leu483Pro is expected to cause the type III GD phenotype, as per existing data about the mutation. Four patients (patient nos. 2, 5, 10, and 11) are on ERT under the temporary bridging therapy program and have shown improvement in general well-being and spleen size. Complete blood counts were available in two of them and it showed improvement in all parameters. Of the remaining nine patients, three (patient nos. 1, 4, and 9) have succumbed due to inability to get ERT, and the rest are not motivated enough for regular follow up.

## Discussion

GD is the most common lysosomal storage disorder. Type I GD accounts for around 95% of cases. Most published literature is focused on Caucasian patients (Mistry et al., 2015). However, there are a few previous studies available from India. In a large case series of LSD from India, GD was the most common and constituted 16% of the total 387 patients with lysosomal storage diseases reported (Sheth et al., 2013). In a study reported from north India, out of ten cases of GD, eight had type I, and one each had type II and type III (Verma et al., 2012). Another study by Nagral et al. reported type III to be constituting 27% of the total analysed 22 GD patients on ERT (Nagral et al., 2011). Of more than 300 mutations documented in GD, p.Leu444Pro(p.Leu483Pro) appears to be the most prevalent in India (Ankleshwaria et al., 2014).

The metabolic defect in GD is a deficiency of acid beta-glucosidase (also known as lysosomal beta-glucocerebrosidase) enzyme (Cox et al., 1997). The gold standard for diagnosis of GD is acid beta-glucosidase enzyme assay in blood leucocytes. However, dried blood spot (DBS) testing is suggested in India as it overcomes the logistical difficulty in sending blood samples over long distances, in good condition (Verma et al., 2015). Plasma chitotriosidase is commonly employed first as a screening marker for the diagnosis of GD and then as a biomarker for monitoring treatment efficacy. A recent study from India (Kadali et al., 2016) has shown that 22% of our population is deficient in plasma chitotriosidase activity. This could explain the normal chitotriosidase level in one of our patients.

GD is not uncommon in Rajasthan. Geographical clustering of GD in a single district suggests the possible need for population screening in that area. Consanguineous marriage appears to be one predisposing factor. The mutation noted in our series i.e., p.Leu483Pro (p.Leu444Pro) is the most common mutation reported from other studies in India. This mutation is most commonly reported to be associated with the subacute neuronopathic type i.e., type III GD. Thus, though all our patients were phenotypically type I GD, their genotype is consistent with type III GD. It would be interesting to see if some

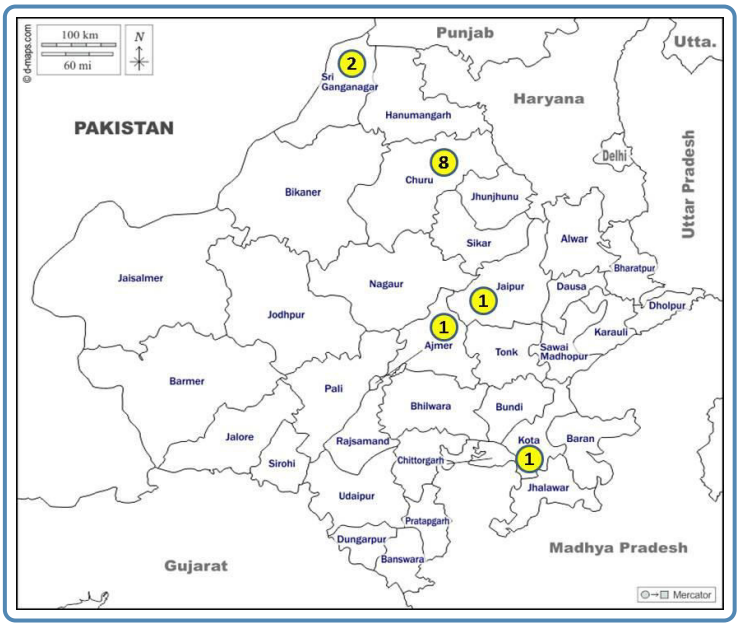


**Figure 2** District-wise distribution of patients with Gaucher disease from Rajasthan in our series.

of these patients indeed progress to the type III GD phenotype in the years to come. We plan to do a repeat detailed neurological and ophthalmological evaluation on follow up, to look for subtle abnormalities seen in type III GD.

One of the main limitations of the present study is that being a study from a tertiary referral centre, the results cannot be generalized to the general population.

This article should help improve the awareness of GD when a child presents with splenohepatomegaly of chronic duration when other causes such as infections, portal hypertension and hemolytic anemia have been ruled out.

**Acknowledgement:** Enzyme replacement therapy for our patients is being provided by Sanofi Genzyme as bridging therapy, on compassionate grounds.

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GeNeXpreSS

# Novel Monogenic Autoinflammatory Diseases

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## Heterozygous *OAS1* gain–of–function variants cause an autoinflammatory immunodeficiency (Magg et al., 2021)

Six unrelated individuals presented with recurrent fever, rash, diarrhoea, failure to thrive and viral-like airway infection. All had normal T cell count, low B cell count and hypogammaglobulinemia. Exome sequencing (ES) identified four *de novo* missense variants (p.Ala76Val, p.Cys109Tyr, p.Val121Gly, and p.Leu198Val) in the *OAS1* gene. OAS1 (oligoadenylate synthetase 1) is a type 1-interferon inducible protein, which initiates an antiviral response against viral double-stranded RNA. Human induced pluripotent stem cell model demonstrated that the gain of function variants in *OAS1* resulted in unprovoked excessive synthesis of a second messenger 2[2032?]-5[2032?]-oligoadenylate (2-5A), which in turn resulted in activated RNAse-L mediated cleavage of cellular RNA, leading to apoptosis of B cells and monocytes. B cell apoptosis caused hypogammaglobulinemia and the dysfunction of macrophages and monocytes lead to activation of pro-inflammatory pathways. This new disorder was named as OAS1-associated Polymorphic Autoinflammatory Immunodeficiency (OPAID).

## Gain–of–function variants in *SYK* cause immune regulation and systemic inflammation in humans and mice (Wang et al., 2021)

The authors ascertained three individuals from two unrelated families. The affected child in the first Chinese family had fever, rash, non-bloody diarrhoea, arthritis, failure to thrive, and perianal fistulas, and succumbed to recurrent infections at 3 years of age. The proband in the second Ashkenazi Jew family developed colitis, arthritis and rash, at 2 weeks of age. Her 35-year-old father also had fever, rash and diarrhoea as a child. ES identified monoallelic variants in *SYK* gene. Patient 1 had a *de novo* c.1649C>A, p.Ser550Tyr variant and patients 2 and 3 had c.1649C>T, p.Ser550Phe variant in *SYK*. SYK is a critical signalling molecule expressed in peripheral mononuclear cells (PBMC), B cells and intestinal epithelium. The variants in the patients resulted in phosphorylation of tyrosine residues at position 525/526 causing constitutive activation of SYK. Similar results were reproduced in transfection studies using HEK293 cells and human SW480 colonic epithelial cells. CRISPR-cas9 edited mice showed that heterozygous mice developed arthritis and bone erosion. The affected mice when treated with a SYK specific inhibitor, R406, showed improvement. Bone marrow transplantation in affected mice showed resolution of disease. This study provides evidence for the role of *SYK* gain-of-function variants in causing inflammation and immune dysregulation in humans and the potential use of SYK as a therapeutic target.

## Somatic mutations in *UBA1* and severe adult–onset autoinflammatory disease (Beck et al., 2020)

ES was performed on three men who had severe inflammatory syndrome with onset between fifth to seventh decades. They had alveolitis, chondritis, and skin lesions, haematological abnormalities like thrombocytopenia, macrocytic anemia and thromboembolic phenomena. A novel, apparently heterozygous variant, at codon 41(methionine-41), in *UBA1* gene, which is on the X chromosome, was identified in them. All three men had a

normal karyotype. Hence, somatic mosaicism was suspected. Sanger sequencing to screen additional patients for variants in *UBA1* identified 25 males with one of the three variants in codon 41 (p.Met41Val, p.Met41Thr or p.Met41Leu). The neutrophils and monocytes of patients had increased proportion of mutant cells. However, mature lymphocytes had more of wild type variant. Transcriptome analysis of peripheral blood cells of these patients showed evidence for activation of innate immune pathways. *UBA1* gene encodes the major E1-activating enzyme, which is required for initiation of ubiquitination signalling. By analysing the expression of UBA1 Met41 variants in human embryonic kidney cells, the authors identified a new isoform of UBA1, which they termed as UBA1c, which had reduced catalytic activity. These findings were recapitulated in the cells from the participants. CRISPR-cas9 edited zebra fish models were used to study the effect of *UBA1* variants on systemic inflammation. The authors thus described a new autoinflammatory syndrome, which they named as VEXAS (Vacuoles, E1 enzyme, X-linked, Autoinflammatory, Somatic).

## Mutations that prevent caspase cleavage of *RIPK1* cause auto–inflammatory disease (Lalaoui et al., 2020)

Seven individuals from three unrelated families presented with recurrent fever and lymphadenopathy, which began in early childhood and continued to adulthood. ES in affected individuals from the three families identified heterozygous missense variants in *RIPK1* gene at the key aspartate residue (p.Asp324Asp, p.Asp324Tyr, and p.Asp324His). RIPK1 (Receptor Interacting Protein Kinase 1) is a key regulator of innate immune signalling pathway like NF-kappa B and MAPK (Mitogen-Activated Protein Kinase) inflammatory pathway. The optimum activity of RIPK1 is maintained by posttranslational modification and caspase-8 mediated cleavage. In normal circumstance, caspase mediated cleavage of RIPK1 prevents activation of inflammatory pathways and necroptosis. RIPK1 cleavage resistant mice model demonstrated that the variants in the key aspartate residue in RIPK1 resulted in resistance to caspase-8 and activation of the proinflammatory pathways. This novel condition was named as 'Cleavage-resistant RIPK1-Induced Autoinflammatory' (CRIA) syndrome.

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