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Carrier Screening and Prenatal Counseling for Inborn Errors of Metabolism: Challenges in the Indian Scenario

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Abstract

Carrier screening for inborn errors of metabolism (IEMs) in the Indian population is challenging because of factors such as consanguinity, inbreeding and inadequate work up done for the index patients. Proper carrier screening is the key to offer accurate prenatal counseling and diagnosis for IEMs. With the advent of next generation sequencing (NGS), it has become fairly easier to offer a definitive diagnosis for IEMs in families. However, there can be many pitfalls in the screening of carriers for IEMs based on NGS methods which have been highlighted in this case series. Eight case scenarios have been discussed, each with a message to highlight the key points that must be taken into consideration while interpreting the results. This will be of immense benefit to all concerned clinicians and counselors who deal with IEMs in the pediatric population and also for those who do antenatal counseling and offer prenatal diagnosis for metabolic disorders.

Introduction

The Indian population is heterogeneous with an admixture of many ethnic and social groups. There is significant consanguinity and inbreeding within the constituent groups with the rate of consanguinity being as high as 30% in some regions, especially in Southern India (Bittles, 2002). This translates into a higher rate of autosomal recessive disorders. Inborn errors of metabolism (IEM) including small and large molecule diseases are easily one of the largest groups of genetic disorders in our population (Kumta, 2005). Studies from the Indian Council of Medical Research (ICMR) and some major genetic centres has put the cumulative incidence of IEMs at approximately 1 in 1500 new-borns (Rama Devi and Naushad, 2004; Kalra et al., 2008). The incidence could be about 1 in 14 neonates if testing is done in a high-risk setting. Therefore, it is expected that the carrier frequency of IEM disorders taken cumulatively is likely to be high (Kapoor et al., 2013).

There are many challenges in diagnosing IEMs in index cases. Though the awareness about detecting and managing IEMs has certainly increased, there are still many lacunae. In many cases the final conclusive diagnosis is not reached because of high early mortality and cost of testing. Protean manifestations of IEM disorders make it difficult to differentiate them from sepsis, cerebral palsy, stroke etc. Hence in many of these cases, metabolic work up is not initiated at all. In many cases critical samples are not worked up or results in some other cases could be misleading because prior medical/ dietary management has distorted the metabolic profile.

NGS- based tests are a big boon for the IEM field, especially in the context of prenatal diagnosis. It is known that testing of biochemical metabolites in the antenatal period may not be very accurate in most cases because it is usually not expected to find a classical metabolic pattern in the antenatal period when the fetus is not adequately metabolically challenged. Similarly, carrier screening of couples for metabolic disorders is difficult at a biochemical level in most cases, except for some X-linked conditions like OTC deficiency, where carrier females may show metabolic derangements when given a metabolic challenge. However, in this case too prenatal testing is not feasible based on metabolites. It is therefore imperative to have a molecular genetic diagnosis for all IEM cases to be able to offer accurate prenatal diagnosis.

With the rampant use of NGS-based tests by many physicians and overreliance only on the results provided by the laboratories without proper reverse phenotyping or parental segregation anal-

ysis, there is likelihood of some misleading conclusions (Green et al., 2013). Many a times couples proceed to bear a pregnancy based on these results without considering a pre-conception consultation with a clinical geneticist or a trained genetic counselor. This can have catastrophic consequences in some cases, especially if the correct diagnosis is not reached. Here we present some case-based examples to highlight the need for proper reverse phenotyping and parental carrier screening before proceeding for prenatal diagnosis.

Case 1

The index child born to 3rd degree consanguineous parents was well till day 3 of life. This was followed by lethargy, poor feeding and seizures and the neonate passed away on the 5th day of life. Metabolic work up revealed raised ammonia, very high citrulline on tandem mass spectrometry (TMS) and presence of traces of orotic acid and uracil on urine organic acid study. The clinical possibility was that of citrullinemia, but the samples of the index child were not available for molecular analysis. Parental carrier screen revealed the following result in both parents in a heterozygous state-

	Gene	Variation	Zygosity
Likely pathogenic variant	*ASS1* (+) Exon 15	c.1168G>A (p.Gly390Arg)	Heterozygous

This variant was classified as a likely pathogenic variant with deleterious predictions as per some bioinformatics tools. It is therefore quite clear after reverse phenotyping that the parents are indeed carriers of citrullinemia, and based on the clinical and biochemical presentation, prenatal diagnosis can be offered for the couple's future pregnancy with a fairly good accuracy.

But in another family, there were 3 early neonatal deaths with similar clinical features as above. However, in the work up of one of the affected children, the ammonia was documented to be normal, amino acid levels were not checked and the organic acid profile suggested only a mild elevation of orotic acid.

One *ASS1* gene heterozygous missense variation of uncertain significance (VOUS) each was detected in both parents of the index case (c.349G>A; p.Gly117Ser). In contrast to the case above, there were many loopholes in proper phenotyping such as a normal ammonia, undocumented plasma amino acids etc. In such cases, prenatal diagnosis may be offered with proper genetic counseling explaining the limitations.

Learning point: A complete biochemical work up is necessary to correlate an NGS test result accurately. Therefore, NGS based testing is complementary and not a substitute to biochemical analysis.

Case 2

Sometimes the testing done in the index case is inadequate, but the clinical pointers and variants obtained in the carrier parents are very strong to suggest a confirmatory genetic diagnosis as in the given case. The index child born of a 3rd degree consanguineous marriage had lost a previous sibling on Day 3 of life with similar features. This child had fever, irritability, lethargy, respiratory distress and seizures. There was significant metabolic acidosis with pH- 7.13, and HCO_3-14.6 meq/L. Ammonia was normal and hemogram showed neutropenia and thrombocytopenia. Sepsis screen was negative. Child passed away before any further work up could be considered.

Carrier screening in parents revealed a likely pathogenic heterozygous variant in the *PCCA* gene in both parents.

The *PCCA* gene showed a 11 base pair deletion in the gene, that was confirmed on qfPCR. Though this variant was not previously reported, it was safely considered for future prenatal diagnosis as it was a deletion variant, likely to result in a frameshift mutation and the clinical phenotype appeared to be consistent with a diagnosis of Propionic acidemia.

Learning point: Gene variant characteristics must be reviewed in each case (Rehm et al., 2013; Richards et al., 2015). These may sometimes be conclusive enough to suggest the diagnosis.

Case 3

In some cases, the clinical possibility of one of the IEMs in the index patient is very strong, but parental carrier screening reveals variants in more than one gene suggesting the possibility of two or more different disorders. This is not an un-

	Gene	Location	Variation	Zygosity
Likely pathogenic variant	*PCCA (+) Exon5*	chr13:100807248_100807258delGCGGATGAGGC	c.316_326delGCGGATGAGG (p.Ala106_Ala109del)	Heterozygous

	Gene/s	Variation observed in both parents	Zygosity
Likely pathogenic variant	*SURF1*	chr9:136219301;G>A, c.751C>T; p.Q251Ter	Heterozygous
Likely benign variant	*DBT*	chr1:100706427;T>C, c.65A>G; p.Y22C	Heterozygous

likely scenario especially in the Indian population because of the high degree of consanguinity and inbreeding.

Clinically the index case had classical features of Leigh syndrome and passed away at 3 years of age. DNA sample of the index child was not available. Parental carrier screening revealed common variants in the *SURF1* and *DBT* genes in both parents.

Based on the clinical phenotype, the *SURF1* gene variant seemed to be significant as it is one of the predominant genes that causes Leigh syndrome phenotype. Also, the *SURF1* gene variant is a nonsense variant with deleterious *in-silico* predictions and thus seemed more likely to lead to a disease state. The other gene namely *DBT* pertains to a phenotype of Maple Syrup Urine Disease. MSUD was not a clinical suspicion in this patient, and a TMS study done in this index case did not reveal any branched chain amino acid elevation. The variant in the *DBT* gene was a missense variation with likely benign predictions. Therefore, for this case the *DBT* gene variant was safely disregarded for future prenatal diagnosis.

But occasionally when a proper clinical/ biochemical/ radiological phenotyping is not done in the index case or if the phenotype pertaining to the given genotype is a late onset one, it may be prudent to consider prenatal diagnosis for more than one variant.

Learning Point: If parents are found to be carriers of more than one disease variant, it is necessary to decide the most appropriate one to offer prenatal diagnosis based on the clinical details and biochemical presentation. It is sometimes not necessary to consider prenatal diagnosis for all the variants detected on NGS testing of families.

Case 4

A 2 years old child born of non-consanguineous marriage presented with mild hepatomegaly and hypoglycemic seizures. Liver biopsy had shown evidence of glycogen storage within the hepatocytes, and hence the possibility of a Glycogen storage disorder (GSD) was very strong. Testing in the index case revealed two heterozygous variants of uncertain significance (VOUS) in the *AGL* gene which pertained to a phenotype of GSD type 6. One variant detected in exon 9 (c.1155G>T;p.Lys385Asn) had deleterious implications on *in silico* analysis and the other in exon 19 (c.2522C>T;p.Ser841Phe) was found to have a 'tolerated' result. It was thought that these variants could have caused the patient phenotype in a compound heterozygous state.

However parental screening showed the result shown in the below table.

Parental studies indicated that both these *AGL* gene variants were monoallelic (see Figure 1) and thus inherited from a single parent by descent, and therefore unlikely to have caused a disease state by themselves. Either the variant in the other allele is a large deletion/ duplication missed on NGS or there is a possibility that this gene is not at all causative of the given phenotype. Further evaluation of the case ruled out GSD and the diagnosis of hyperinsulinemia was confirmed.

Patient Name	Gene studied	Variation observed in proband	Status
Father	*AGL* (Exon 9)	chr1:100340782G>T (HET); c.1155G>T; p.Lys385Asn	Detected Heterozygous
	AGL (Exon 19)	chr1:100349983C>T (HET); c.2522C>T; p.Ser841Phe	Detected Heterozygous
Mother	*AGL* (Exon 9)	chr1:100340782G>T (HET); c.1155G>T; p.Lys385Asn	Not Detected
	AGL (Exon 19)	chr1:100349983C>T (HET); c.2522C>T; p.Ser841Phe	Not Detected

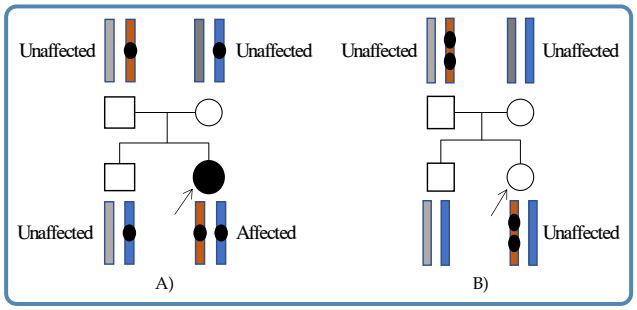

Figure 1 A) Biallelic variants in the same gene can cause a disease (Compound heterozygous), B) Monoallelic variants cannot cause a disease by themselves.

Learning Point: Confirmation of carrier status in the parents is absolutely necessary even if the index case has been apparently diagnosed with a fair degree of genotype-phenotype correlation. Occasionally this step may be circumvented for various reasons, the most important being the cost and time, but this can have disastrous consequences as seen in the above case.

Case 5

Occasionally the phenotype in the index case is unambiguous, but more than one variant may be noted in the same gene. Parental screening resolves the conflict and aids in offering an accurate prenatal diagnosis. As in this case with Wolman disease there were 2 variations noted in a homozygous state in the index case.

Both the above variants were classified to be those of uncertain significance (VOUS). The exon 2 variant in the *LIPA* gene is present in a homozygous state in the unaffected parents, but the exon 4 variant is noted in heterozygous state in the parents. Thus, based on the variant and segregation analysis, prenatal diagnosis was offered only for the *LIPA* gene exon 4 variant.

Learning Point: Parental segregation analysis resolves the conflict between likely pathogenic and likely benign variants in cases where more than one variant is noted in the same gene.

Case 6

In a clinically, biochemically and radiologically proven case of Succinic semialdehyde dehydrogenase (SSADH) deficiency in a non-consanguineous family, 2 pathogenic variants were noted in the *ALDH5A1* gene. It seemed that the two different variants would have caused SSADH deficiency in a compound heterozygous state. But parental analysis showed presence of both these pathogenic variants in the father and none were present in the mother, thus suggesting monoallelic inheritance by descent.

Further tests were considered in the mother by multiplex ligation-dependent probe amplification (MLPA), which revealed heterozygous deletions in exons 3 and 4 of the *ALDH5A1* gene. This explained the mechanism of disease causation in the proband.

Learning Point: Sometimes very obviously pathogenic looking variants can be extremely deceptive. Additional studies with some alternate methods may be required after segregation analysis to determine the variants causative of the disease.

Case 7

The index child born of 3rd degree consanguinity, was clinically suspected to have Leigh disease with history of neuroregression, jerky breathing, ptosis and seizures. The child passed away and no samples of the deceased child were available for testing. Parental testing initially revealed a heterozygous missense variant of uncertain significance in the *PDHX* gene which pertained to a phenotype of Pyruvate Dehydrogenase E3 binding protein deficiency. Clinically and biochemically however the index child did not fit into the *PDHX* gene- associated phenotype. Hence, the couple

Parent	Gene / Exon	Variation observed in proband	Status
Father	*LIPA* (Exon 2)	chr10:91007360T>G(HOM); c.46A>C;p.Thr16Pro	Homozygous
	LIPA (Exon 4)	chr10:90988077C>A (HOM); c.308G>T; p.Ser103Ile	Heterozygous
Mother	*LIPA* (Exon 2)	chr10:91007360T>G (HOM); c.46A>C; p.Thr16Pro	Homozygous
	LIPA (Exon 4)	chr10:90988077C>A (HOM); c.308G>T; p.Ser103Ile	Heterozygous

Gene	Variation	Father	Mother
ALDH5A1 (+) Exon 3	c.467_480delinsTGT;p.Glu156ValfsTer10	Heterozygous Pathogenic	Absent
ALDH5A1 (+) Exon 5	c.813_819delinsCTGGTGTAG;p.Cys272TrpfsTer30	Heterozygous Pathogenic	Absent

were counseled that no prenatal diagnosis would be possible based on the detected variant. The couple chose to wait, and data reanalysis was done after a gap of 2 years. When the data was reanalysed using the revised pipelines, a heterozygous pathogenic deletion of exons 3 and 4 of the *NDUFS4* gene was suspected, which was later confirmed by MLPA. Therefore, prenatal diagnosis could be offered for the family for Mitochondrial Complex 1 deficiency. This was possible because of periodic data reanalysis which sometimes can reveal novel variants.

Learning Point: Prenatal diagnosis should never be offered for variants where the genotype does not correlate with the phenotype in the index case. This case highlights the need for periodic reanalysis of the data if no significant variant has been detected earlier.

Case 8

A child born of 2nd degree consanguinity was clinically suspected to have malignant infantile osteopetrosis based on the classical clinical features. NGS (exome) based testing revealed a homozygous pathogenic variant in the exon 4 of the *OSTM1* gene (c.721dupA; p.Met241AsnfsTer3) that seemed likely to have caused the given phenotype. Parental studies showed presence of the same variant in a heterozygous state in the maternal sample, but this variant was not seen in the paternal sample. The paternal sample was retested again to rule out any false negatives. This was followed by paternity testing on the sample of the index case. This test revealed that the father was not a biological parent of this child. Thus prenatal diagnosis cannot be offered based on this information and analysis of whole *OSTM1* gene in biological father will be needed.

Learning Point: Cases such as these highlight some unique challenges that are faced during carrier screening of apparently simple cases.

Testing of the mitochondrial genome is even more complex because of factors such as heteroplasmy and tissue mosaicism that cause problems with accurate genotype- phenotype correlations. Similarly, blended phenotypes must be meticulously evaluated to be able to reach a conclusive diagnosis.

Conclusions

The above case series highlights the issues that are faced in carrier screening in the absence or sometimes even in the presence of a definite diagnosis in the index patients. It is necessary to evaluate all suspected IEM cases as thoroughly as possible with relevant biochemical and radiological studies. In cases with impending bad outcomes, DNA must be stored for future use. Pre-conception evaluation of the couple by a Clinical Geneticist is necessary in all cases where prenatal diagnosis has to be offered. Evaluation of the index case (if available), thorough review of the NGS test data performed in the index case and parents, proper reverse phenotyping with clinical, biochemical and radiological presentations are all essential and mandatory steps to confirm the diagnosis to be able to offer an accurate prenatal diagnosis.

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