Case Report

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Maple Syrup Urine Disease - Role of Next Generation Sequencing, A Newer Molecular Technique

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Abstract

Maple syrup urine disease (MSUD) is an inborn error of metabolism caused by defects in the branched-chain α-ketoacid dehydrogenase complex, which results in elevations of the branched-chain amino acids (BCAA) in plasma, α-ketoacids in urine, and production of the pathognomonic disease marker, alloisoleucine.

The classic presentation occurs in the neonatal period with developmental delay,failure to thrive, feeding difficulties, and maple syrup odour in the cerumen and urine, which can lead to irreversible neurological complications, including stereotypical movements, metabolic decompensation, and death if left untreated.

Most important challenge is to diagnose a disease condition which presents with wide range of clinical symptoms or may be in cases being subclinical without any manifestations. Even though biochemical tests give us precise report to diagnose MSUD, mutation studies by sequencing genome helps in early detection of disease or carrier status thus, helps in proper management. As age advances, switching over to newer modality of investigations is necessary, like sequencing the exomes carry more weightage than sequencing whole genome, which is cumbersome and time consuming. Here, we are discussing the cases which were diagnosed using Next generation sequencing (NGS).

Keywords: Maple Syrup Urine Disease, Branched Chain Aminoacids; Next Generation Sequencing; Mutation.

Introduction

Branched chain organic acidurias are a group of disorders that result from an abnormality of specific enzymes involving the catabolism of branched chain aminoacids (BCAAs) i.e., Leucine, Isoleucine, Valine wherein the catabolic pathways of BCAAs were blocked at the decarboxylation of the respective á-ketoacids [1]. Therefore, the disease is alternatively known as "Branched chain ketoaciduria" or "Branched chain ketonuria" [1]. The condition gets its name from the distinctive sweet odour of affected infant's urine which smells like maple syrup (caramel syrup).

This condition is inherited in an autosomal recessive pattern, which means both copies of the gene in each cell have mutations. MSUD affects males and females in equal numbers. The incidence of MSUD is estimated to be around1 in 2,00,000 live births [2].

Pathogenic homozygous or compound heterozygous variants in BCKDHA, BCKDHB, DBTor DLD, which form the catalytic subunits of branched-chain α-ketoacid dehydrogenase (BCKAD) complex, can result in MSUD [1]. This disorder is characterized by neurological and developmental delay, encephalopathy, feeding problems, and a maple syrup odour to the urine due to accumulation of BCAAs [2].

MSUD can be diagnosed by clinical, radiological and laboratory findings. Genetic testing aids in confirming the diagnosis by unravelling mutations, also determining the carrier status. The recent technique used for interrogating genetic mutations includes next-generation sequencing (NGS).

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Here, we are presenting two cases which were screened using newer technique, NGS and helped in appropriate management to reduce morbidity/ mortality.

NGS, is high-throughput sequencing that allows us to sequence Deoxyribonucleic acid(DNA) and Ribonucleic acid (RNA)at a faster pace than Sanger sequencing. Moreover, it is more economical than Sanger sequencing [3]. The principles at the core of NGS, which galvanized its drastic increase in throughput over the traditional Sanger sequencing, are DNA amplification without bacterial cloning and DNA sequencing without chain termination. Each fragment of DNA is PCR-amplified independently, in a way that the amplification products are spatially clustered. Sanger's terminator technique is replaced by sequencing either by synthesis or by ligation [4].

NGS can bring to light a wider spectrum of mutations as compared to Sanger sequencing and genomes can be probed without bias as NGS is unselective and scrutinizes full genomes or exomes to discover novel mutations and genes causing disease. This could be exploited to gather information about the genetic basis of unexplained syndromes [5].

Case Report

Case 1

A healthy couple, wife being 4 months pregnant walked into our genetic clinic with the history of loss of two children affected with MSUD, born of consanguineous marriage. Both children were diagnosed with this condition based on clinical presentation and Magnetic resonance imaging (MRI). Evaluation for the carrier status of the couple to yield a healthy baby was necessary. Thus, mutation study by Clinical exome sequencing usingNGS was done after obtaining consent.

NGS was done only for the MSUD panel and showed BCKDHB mutation in both parentsat exon 5 on chromosome6. Moreover, it was autosomal recessive disorder with heterozygosity.

Hence, posttest genetic counselling was done and the couple was intimidated about the fact that there is a 25% chance of children being affected in the subsequent pregnancy and also advised for prenatal screening of the subsequent pregnancy for the known mutation.

Case 2

A male baby aged 3years, born of a consanguineous marriage, first child of the couple, manifested with meningitis, neonatal seizures and developmental delay. MRI showed restricted diffusion involving bilateral cerebellar white matter, entire brain stem, internal capsules and corticospinal tracts which was suggestive of MSUD.

To analyse for the pathogenic variant NGS was done on the baby which showed the mutation in DBT gene on chromosome 1 (exon 2-3 deletion) which was homozygous and autosomal recessive in inheritance.

Estimation of aminoacids and acylcarnitine by Tandem Mass spectrometry was done which depicted elevated Valine, Leucine and Isoleucine along with Hexadecenoylcarnitine (C16) and Stearoylcarnitine (C18).

In our case series, clinical exome sequencing was done using illumina sequencing platform. For library preparation genomic DNA isolated from blood (Saliva or any other tissue can also be used) was used to make library. The DNA was quantified using a Qubit and 50ng was taken for library preparation. TruSight One library preparation uses transposon based shearing of the genomic DNA, the protocol allows the DNA to be fragmented and tagged simultaneously in the same tube. A limited cycle PCR step allows the incorporation of adapters, platform-specific tags and barcodes to prepare the DNA sequencing libraries. During target enrichment, the tagged and amplified sample libraries were checked for quality and quantified. 500ng of each library was pooled into a single tube and subjected for enrichment using biotinylated, target specific probes. Target libraries are amplified using limited PCR steps and loaded for sequencing on the illumina MiSeq/NextSeq. Pathognomonic variations in BCKDHB and DBT genes were identified using the NGS software.

Discussion

Many infants with MSUD are identified through newborn screening programs. Tandem mass spectrometry is an advanced new born screening test that screens for more than 30 different disorders through one dried blood spot sample from heel prick. In certain situations where testing for MSUD is not available or where Newborn Screening (NBS) fails to detect MSUD, a diagnosis may be suspected based upon symptomatic findings. But, to arrive at a firm diagnosis we need an accurate and specific investigation.

MSUD is mainly inherited as autosomal recessive pattern where the risk for two carrier parents to pass the altered gene and have an affected child is 25% with each pregnancy. The risk to have a child who is

a carrier like the parents is 50% with each pregnancy. The chance for a child to be normal without being carrier or affected is 25%. The risk is the same for males and females.

Parents who are close relatives (consanguineous) have a higher chance than unrelated parents to carry the same abnormal gene, which increases the risk to have children with a recessive genetic disorder. Thus, awareness among people is needed about the more incidence of the disease in consanguineous marriage.

Meanwhile, molecular genetic testing for mutations in the above-mentioned genes plays an important role in confirming the diagnosis, and is necessary for the purpose of carrier screening for at-risk relatives and prenatal diagnosis for at-risk pregnancies. Mutation study will also help to categorise the disease and also helps to understand the course of the disease.

The information provided by the MSUD mutations will allow prenatal and carrier detection at the gene level. As more MSUD mutations are identified,their origin and population distribution can be addressed. Moreover, the mutations in different subunit of the BCKAD complex appear to be useful genetic models to study the effect of MSUD mutations on macromolecular organisation and protein-protein interactions [2]. It is anticipated that the progress made in understanding the molecular basis of any inborn error of metabolism will ultimately lead to the development of effective gene therapy for the same.

Early diagnosis and treatment stabilizes the infants and, if well performed, can largely mitigate against serious metabolic decompensations and long-term complications. Treatment often begins with aggressive intervention in an acute metabolic crisis. The levels of organic acids and branched chain amino acids in the plasma can be lowered by haemodialysis. Adequate administration of intravenous (IV) fluids can enhance excretion of organic acids by renal loss. IV glucose serves as an alternate source of energy and diminishes protein catabolism, thus decreasing the branched chain amino acid production in acutely ill infants. Lifelong prohibition of protein intake which is rich in branched chain aminoacidsis essential. Commercial formulas devoid of branched chain amino acids are available. Repletion of carnitine stores and removal of organic acid can also be aided by carnitine supplementation. It has been noticed that some patients are responsive to high doses of thiamine supplementation which could be effective in infants, who are critically ill. Patients who survive the newborn period or who have a presentation later in life needs close monitoring to prevent morbidity and mortality. Even though, MSUD can be diagnosed by biochemical investigations at the onset of symptoms, genetic testing can be considered superioras it assists in prenatal diagnosis of the carrier or disease state, which in turn would result in early awareness and better management even before the onset of precarious affects.

Hence, the application of new technique such as NGS will be important to evaluate the cases and to aid in management.

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