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RESEARCH NOTE

Prenatal identification of a novel mutation causing methylmalonic acidemia in a family without proband

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Introduction

Methylmalonic acidemia (MMA, MIM 251000) is an autosomal recessive genetic disorder that occurs in about 1 in 50,000–100,000 people (http://ghr.nlm.nih.gov/condition/methylmalonic-acidemia). It is characterized by buildup of methylmalonic acid in the body. It was first reported in London from Queen Elizabeth Hospital for Children. The condition is caused due to the inability of the body to convert methylmalonic acid to succinic acid (Oberholzer *et al.* 1967). This might be due to partial absence of the necessary enzyme, methylmalonyl-CoA mutase, termed as mut (-) or complete absence of the enzyme, depicted as mut (0).

MMA is an early onset disease, usually seen in infants but it can also develop later in adulthood. Symptoms of MMA include vomiting, dehydration, hypotonia, lethargy, seizures, respiratory distress, intellectual disability etc. The nonspecific nature of these symptoms makes accurate diagnosis difficult (Alireza Radmaesh 2008). Also, the severity of the disease can vary from being benign to fatal (Matsui *et al.* 1983; Ledley *et al.* 1984) and is further complicated by the patient's response to treatment with vitamin B12 (Morrow *et al.* 1969). Abramowicz *et al.* (1994) was able to determine the locus of its causative gene to be as chromosome 6, while Jansen and his colleagues were first to identify the mutations on the *MUT* gene (Jansen and Ledley 1990; Abramowicz *et al.* 1994).

In this study, we report a novel mutation, V733I, in a family affected by this disorder but proband was not present at the time of testing. We therefore used sequencing analysis to determine the carrier status of parents before offering prenatal testing for the following two pregnancies.

Case history

A consanguineous couple, female 32 and male 35 years (figure 1, VI-2 and V-7) was referred to our genetic counselling centre with the history of losing two infants. Clinical diagnosis was suggestive of metabolic disorder, methylmalonic acidemia (MMA). The couple were anxious to conceive and wanted to understand the risk for future pregnancies, and also possibilities of prenatal diagnosis.

Pedigree analysis of the family obtained during counselling showed high incidence of consanguinity in the immediate and extended family. As seen in figure 1, male partner (V-7) is the maternal uncle of female partner (VI-2) and parents (IV-2 and IV-3; IV-1 and V-1) of both the partners were also consanguineously related. Female partner had lost two male siblings (VI-1 and VI-3) between 8 and 9 years of age.

Although no medical reports are available to verify, vomiting, growth retardation and epilepsy were common clinical symptoms reported in both the deceased siblings. Male partner also had lost one male sibling (V-4) and two female siblings (V-3 and V-5) with similar presentation. His mother also had three additional pregnancy losses in first trimester (data not shown). Family history reveled a few other incidents of neonatal loss (all marked as NND in figure 1).

The couple had their first male child (VII-1) within one year of marriage. The child was born by C-section with asymptomatic, within normal weight and no dysmorphology. The child was growing normally. Symptoms like lethargy, poor feeding, dehydration hypothermia, vomiting developed soon after the infant was introduced to solid food. The condition progressed rapidly to coma stage and the child died within 12 months. The copule had their second child, female (VII-2) born after 2 years and had the same symptoms as the first child and expired around 12 months of age, secondary to fulminant sepsis.

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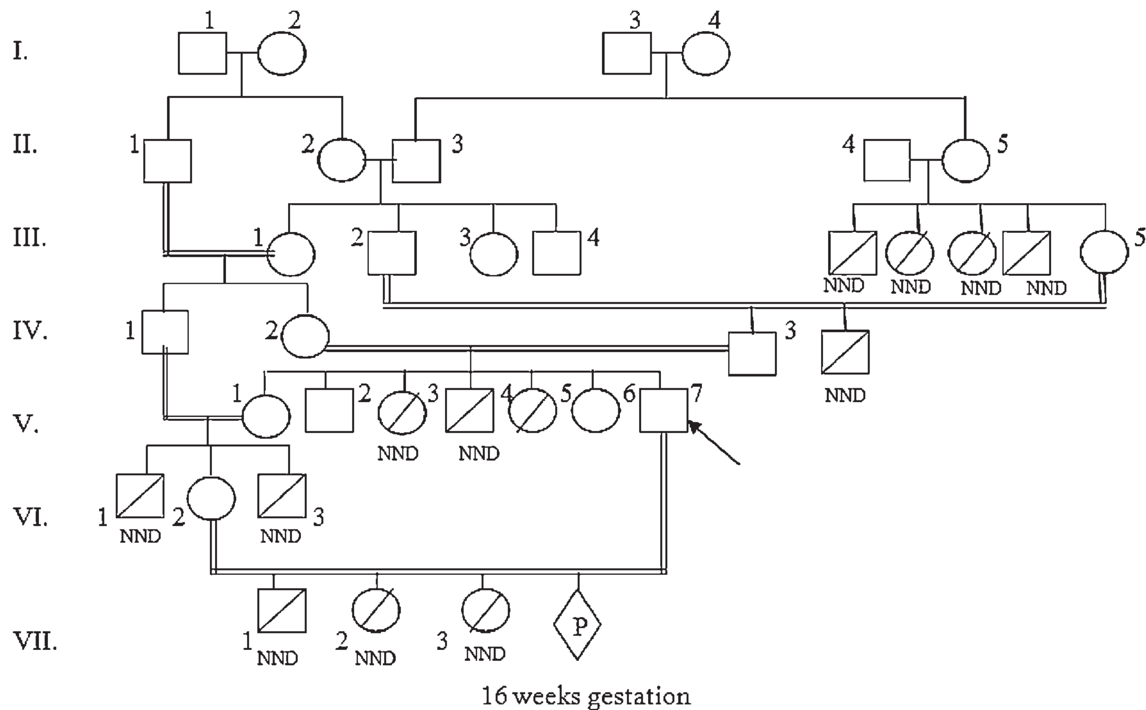

Figure 1. Pedigree of the family showing high incidence of consanguinity for three generations. NND, neonatal death; arrows indicate partner first tested in our lab. Symbols: square, male; circle, female; square and circle with line across, deceased individual; diamond, pregnant.

Although no medical reports were available for the first child, the reports of second child showed severe ketoacidosis and hyperammonia, high concentration of methylmalonic acid in urine along with ketoaciduria (Urine organic acid analysis) and sepsis-induced lactic acidosis. However, no significant primary lactic acidosis was noted.

Reports of the partners showed no sign of organic aciduria with normal levels of urinary oritic acid. Orotidine, urine creatinine and ratio of urinary orotic acid, as revealed by their Urine GCMS profile. Karyotyping of both partners showed normal 46,XX and 46,XY karyotype without any numerical or structural defects.

Medical reports of the child were highly suggestive of MMA. However, standard confirmation of diagnosis by measuring propionate incorporation of methylmalonyl CoA mutase activity was not done. Thus, the couple was counselled assuming that the infants could have been affected with MMA. In the absence of proband, carrier testing for *MUT* gene mutations was suggested to determine the disease causing mutation in the family. An Informed consent was obtained from the couples.

Table 1. Primers sequences and the annealing temperature used for exons.

Exon	Forward primer	Reverse primer	Annealing temp. (°C)
Exon 1	GCTTAGAGTAATTCGCGCCC	TAAAGGACAGAGCGGGAGAG	56
Exon 2	AGGAAGCAGAAAAGGGGAAG	CACCTAATTCCTTTTAAACAACGTG	55
Exon 3	GCACAAGGGAAACAACTGAC	AGATCTGTTGCCCAGATTCC	55
Exon 4	AGTCCTGATGATGGTTCATGG	AAGATATTCCTCAAATTTACAAAGAAG	55
Exon 5	TTGCTGTTTTATTTCATTCATAGG	TTTCAGCACTACAGGGAAGC	57
Exon 6	GAACTCTGACTCTTCTAATTTTGGC	AAAACTGCTGTTCTTTGTATGAGC	56
Exon 7	TGATGTTGCTTTAATTTTCTCCC	TATGCTTGCCTGTGTGCATC	57
Exon 8	TTCTCAGATTGGGATTTGCTG	CACACCTCATGCTGTTGTAAGG	55
Exon 9	TTTTCCTCTTAAATTTGGAGTTAGG	GGGCTCACATGGTTTACAGG	55
Exon 10	GAGAATTGGATGCATAAAGGC	TCTCAGTTGTATGTAAGGAAATTAAGC	56
Exon 11	AAGATTTGCTGTGAATAA	TGTCATCATTTTACTACATT	53
Exon 12	AATAACTTGGGCTGGGAGGCTG	TGTACATACTTATAGCATGACACCAGG	65
Exon 13_1	CCAGTTGAGAAGGTTTTGGG	TGGATTTTATTTTCTGGTGCTC	56
Exon 13_2	TTTATCAAGAACTCTGGACAATGG	GGTAAATGGCAACTTTCCTTC	56
Exon 13_3	TGCTACCTTGCATAGATTTGCTC	CTGACTTCGGGTAAACACACAC	57

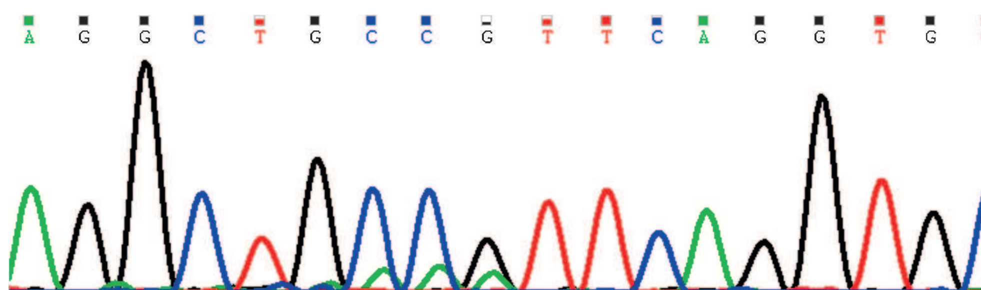

Figure 2. Heterozygous change in nucleotide G→G/A, leading to V733I mutation in *MUT* gene.

Material and methods

The *MUT* gene, located on the 'p' arm of chromosome 6, consists of 13 exons. Primers of all exons for this gene were designed using Exon Primer software (from http://ihg.gsf.de/ihg/ExonPrimer.html), except in the case of exon 12, which were designed manually. All primer sequences are listed in table 1. PCR reactions consisted of 2 nM dNTPs (Cat BIO-39025, Bioline, UK), 2.5 mM Mg^{+2} ions, 0.5 μM each of forward and reverse primers (obtained from Eurofins Genomics Pvt. Ltd. Begaluru, India) and 0.2 units of BIO-Taq Red (Cat BIO-21041, from Bioline, UK) were then standardized to amplify these exons individually. Annealing temperature used for the PCR varied depending on the melting temperature of the primer. Primers sequences and annealing temperature used for each exon are tabulated in table 1. Amplified PCR products were then purified using QIAquick PCR Purification kits (Cat 28104, Qiagen, Germany) as per the manufacturer's protocol followed by bidirectional Sanger Sequencing on ABI 3730 Xl Genetic Analyzer (Applied Biosystems, Foster City, USA).

Results

All sequencing results were analysed using Mutation Surveyor, DNA variant analysis software from SoftGenetics LLC, USA (http://www.softgenetics.com/mutationSurveyor.html). Analysis of PCR-sequenced exons revealed a heterozygous change from nucleotide G to A in exon 13 resulting in amino acid change from valine to isoleucine leading to a missense mutation at amino acid position 733 and thereby conferring carrier status to one partner (see figure 2).

The other partner was also tested and found to be a carrier of the same mutation, thereby confirming the disease causing mutation in the family. To the best of our knowledge, this is the first instance where the V733I mutation has been reported in association with MMA.

Genetic counselling

During counselling secession, the risk of recurrence was explained to the family to be as 25% since both parents were carriers of the mutation. Later after a year the couple conceived and approached our centre for prenatal diagnosis. Amniocentesis was done at 16 weeks of gestation, amniotic fluid was cultured and then tested for the familial mutation as well as karyotyping. Both karyotyping as well as PCR-sequencing analyses for the familial mutation showed normal results. Therefore, it was concluded that foetus is unlikely to be affected with MMA. The pregnancy was continued and a normal child was delivered. Routine Newborn screening by Tandem Mass Spectrometry showed normal organic acid levels in the new born. The child is now about three years old and healthy. The couple conceived again and prenatal diagnosis again revealed likelihood of normal foetus unaffected with MMA.

Discussion

Since Jansen and Ledley's first reported heterozygous mutations in a patient with MMA (Jansen and Ledley 1990), there have been 357 variations (http://www.ensembl.org/Homo_sapiens/Transcript/ProtVariations?db=core;g=ENSG00000146085;r=6:49430360-49463191;t=ENST00000274813) that have been reported or predicted in the *MUT* gene. However, the mutation V733I that was found in this study has not been reported earlier.

Testing for common mutations in the *MUT* gene would have not revealed this novel mutation in the family, thereby denying the family the opportunity to make an informed decision. Such a finding emphasizes on the need to treat each case as unique and carry out detailed testing, wherever possible and is especially true in case of rare disorders such as MMA. Further, advances in medical treatments now allow patients with MMA to lead a normal life (Wasserstein *et al.* 1999). Therefore, clinicians must use every possible opportunity to detect such disorders as early as possible for the patient so that dietary modifications can be brought into place immediately and allowed the foetus to develop normally.

This report also shows the importance of genetic counselling for families affected with genetic disorders and application of DNA sequencing technology in medical diagnostics. Even in the absence of the proband, sequencing technology can reveal mutations in carrier parents (in case of autosomal recessive conditions) and provides us valuable information for disease management. This technology can also

be used to determine accurately the presence or absence of mutation in the foetus and can facilitate appropriate decisions for the family.

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