Mutation Analyses in Selected Exons of the MUT Gene in Indian Patients with Methylmalonic Acidemia

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Abstract Deficiency or diminished activity of a cobalamin dependent enzyme methylmalonyl-CoA mutase causes inborn error of metabolism called methylmalonic acidemia (MMA). In this study we elucidated the spectrum of mutations in 21 Indian mut MMA patients by direct sequencing. Sequence analysis identified a total of 70 mutations in exon 2, 9, 11 and 12 of MUT gene. Out of which 26 mutations were predicted to be deleterious and rest were benign. The 23 novel mutations consist of four nonsense mutations (p.N6*, p.G539*, p.E609* and p.I671*), twelve missense mutations (p.K128I, p.N547T, p.D554Y, p.A558T, p.R559P, p.A631T, p.I647T, p.E656D, p.V657E, p.Q660H, p.K679N, and p.G696Y) and seven frame shift mutations (c.375_376insA, c.1642delA, c.1655delC, c.1825_1826insT, c.1957delGA, c.2014delA and c.2062_2063insGA). All of them are point mutations or micro rearrangements. Three of these mutations (p.K621N, p.G648D, p.G630E) have been previously reported; all of them are missense mutations. The mutations are distributed throughout the exon 2, 9, 11 and 12, 38.4 % mutation are located in exon 12.

Keywords MUT gene · Methylmalonic acidemia · Mutation analysis · GCMS · LCMSMS · Vitamin B12 deficiency

Introduction

Methylmalonic acidemia (MMA) is the commonest organic acidemia in which the concentration of methylmalonic acid is massively increased in the blood, urine and cerebrospinal fluid (CSF). Approximately 60 % cases of MMA are caused due to the mutation in MUT gene which causes mut^o variant of methylmalonic acidemia (MMA; MIM # 251000), an autosomal recessive disorder. It is caused by inherited defects in the methylmalonyl-CoA mutase enzyme (MCM) (EC 5.4.99.2). The MUT gene encoding this enzyme has been mapped to chromosome 6p12–21.2 [1]. It contains 13 exons spanning over 35 kb of genomic DNA [2] and is transcribed in the nucleus as a 2.8-kb mRNA. In mitochondrial matrix, 32 amino acid mitochondrial leader sequence is cleaved by a peptidase to form mature subunit (718 amino acids), two subunits then assemble to form a homodimer with ~78 kD subunits that binds two molecules of adenosylcobalamin (AdoCbl) to form the active holoenzyme [3, 4].

There have been reports of specific mutations among various ethnic groups or hotspots, including p.G717V in African-Americans [5] and p.E117X in Japanese patients [6]. Six mutations p.L494X, p.R93H, p.E117X, p.R369H, p.G648D, and c.385 + 5G>A were identified in another Japanese population [7]. The mutation p.N219Y was identified in five unrelated families of French and Turkish descent as well as in other populations [8] and p.R108C has been noted in Hispanic individuals [9]. Mutation p.R369C occurs in diverse populations [10]. A splice-site mutation IVS8 + 3A→G recurs in the Arab-Moslem population [11]. Worgan et al. identified exons 2, 3, 6 and 11 as mutation clusters while Acquaviva et al. found 67 % mutations were located in exon 2, 3, 11 and 12 [8, 12]. Studies on Indian patients are scarce in published literature.

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We sequenced exon 2, 9, 11 and 12 of the MUT gene to detect the hotspot of *mut*^o variant of MMA patients and to examine the spectrum of mutations in these specific exons in our population. Structural modeling was also done to elucidate the effect of substitution in the structure of protein.

Materials and Methods

In this study, 21 apparently unrelated MMA patients were studied. All patients with available clinical information had been symptomatic during their neonatal or infantile periods. There were no consanguineous marriages among the parents of these patients. The diagnoses of Methylmalonic acidemia were confirmed by presence of highly elevated urinary methylmalonic acid by gas chromatography mass spectrometry. These diagnoses were re-confirmed using tandem mass spectrometry (LCMSMS) by seeing elevated level of propionylcarnitine (C3), C3:C2 levels and methionine in patients dried blood spots. However no enzyme assay was available to confirm the diagnosis. For mutation study, we collected blood samples of patients in a EDTA containing tube. The Institutional Ethical Committee (IEC) of Maulana Azad Medical College, New Delhi has approved this study and consent of parents of the patients has taken before the study was carried out.

Direct Sequencing of the Amplified DNA

The genomic DNA was extracted from the two ml venous blood of the patient by phenol chloroform method [13]. The genomic DNA isolated was used for amplification of exon 2, 9, 11 and 12 of MUT gene using PCR method. For every DNA sample four 50 µl PCR reaction mixture were prepared containing specific set of primers for amplifying the desired exon. The reaction mixture in every tube

contained 1 X PCR buffer containing 2.5 mM MgCl₂, 0.2 mM dNTPs (nucleotides), 0.2 µM of each primer, 0.2-U Taq DNA polymerase, and 0.5 µg of DNA. Samples were overlaid with 50 µl mineral oil and subjected to amplification in a thermal cycler. A four step PCR was performed in a thermal cycler using specific PCR conditions. Table 1 showing the sequence of primers used for the amplification along with their annealing temperature. The band size and amplification of PCR products were evaluated by agarose gel electrophoresis (Fig. 1). Then PCR products were directly sequenced using Applied Biosystems 3730 DNA Analyser. Both forward and reverse sequencing of the target exons were carried out to rule out any sequence artifacts. The sequences were then explored using the Geospiza's Finch TV and NCBI nucleotide BLAST (Basic Local Alignment Search Tool). Figure 2 showing exon and intron showing various mutations and the partial nucleotide alignment of the exon 12 of MCM for the studied patients.

We screened 40 controls for mutations in order to distinguish between polymorphism and mutation in our population. The common mutations and variants reported so far were filtered using Exome aggregation consortium (ExAc) data and the human gene mutation database (HGMD). The mutation nomenclature used in this study follows the guidelines of den Dunnen and Antonarakis [14] using GenBank accession no. NG_007100.1 as the reference genomic sequence that cover MUT transcript NM_000255.3.

Genotypic and Phenotypic Correlation

It is very important to find out the genotypic and phenotypic correlation of these mutations to classify them as benign or potentially deleterious. Here we used three softwares PolyPhen-2, PROVEAN and SIFT for predicting the effect of mutation in the proteins structure and function [15–17]. HumVar dataset was used to predict the effect of

Table 1 Showing the sequence of primers along with their annealing temperature and band size of each exon

Exon	Primer no.	Sequence	Anneal temp. (°C)	No. of cycles	Band size (bp)
2	112	5'-CAG GGT TTT TAT AGT CAT TA-3'	54	30	500
	70*	5'-AGC TCC TAT TCC ACC CCT CTT C-3'			
9	9-1	5'CCTTTCCTTGACTTTTTC3'	53	40	250
	9-2*	5'TGCTGTCATCATTTTACTAC3'			
11	11-1	5'ACTTGAAGATTTGCTGTG3'	48	30	325
	11-2*	5'TGCTGTCATCATTTTACTAC3'			
12	12-1	5'CAGGGTTTTTATAGTCATTA3'	49.1	30	250
	12-2*	5'CAAGATTCCCATCACAGT3'			

* Reverse primer

Fig. 1 Gel picture of amplified exons (2, 9, 11, 12) and ladder of 50 bp showing the band size. M = 50 bp ladder molecular marker, lane 1 exon 2, lane 2 exon 9, lane 3 exon 11 and lane 4 exon 12

substitution on structure of protein analyzed by PolyPhen-2 software. It consists of all the 13,032 human disease causing mutations from UniPort and 8946 human non-synonymous single nucleotide polymorphisms (nsSNPs) without annotated involvement in disease, that were treated as non damaging [17]. Mutation taster (<http://www.mutationtaster.org/>) was used for analyzing nonsense and silent mutations [18].

Structural Modeling

To determine the effect of mutations in protein structure, three dimensional structural modelization was done. For this sequence files are processed with sequence analysis software and all the illustrations have been prepared using molecular visualization software Pymol.

Results

All the patients with available clinical information had been symptomatic during their neonatal or infantile periods. As initial clinical findings, symptoms such as global developmental delay (63 %), lethargy (48 %), vomiting (37 %) and seizures (37 %) were most frequent followed by poor feeding and hypotonia which were relatively common (26 % respectively). Family history of a similarly affected sibling was noted in 4 % cases. The most common laboratory finding at the onset of disease was abnormal lactate (37 %) followed by hyperammonemia (30 %), abnormal MRI (26 %), acidosis (11 %) and ketosis (7 %).

The diagnoses of Methylmalonic acidemia were confirmed by elevated levels of methylmalonic acid in urine in GCMS analysis. These diagnoses were re-confirmed using LCMSMS by seeing elevated level of propionylcarnitine (C3), C3/C2 ratio and methionine levels in patients dried blood spots (Table 2).

Out of 21 patients whose DNA sample has been sequenced 19 patients had deleterious mutations; two

patients had only benign mutation. It is likely that deleterious mutations are present in some other exons or non-coding or regulatory regions of the MUT gene in these two patients. The biochemical results for all the patients were indicative of mutase deficiency.

A total of 70 mutations were observed in the four exons. 12 (17.1 %) out of 70 were silent mutation. 38 (54.2 %) mutations are missense mutation. 16 (22.8 %) mutations accounted for frame shift mutations that are caused due to deletion or insertion of the nucleotide. Only 4 (5.7 %) mutations were nonsense mutations. Out of 70 mutations 37.1 % (26 of 70) were predicted probably damaging and rest were predicted benign. Most of the mutations that have deleterious effect on phenotype are located in exon 12 (38.4 %, 10 of 26) followed by exon 9 (26.9 %, 07 of 26), exon 11 (23.0 %, 06 of 26) and exon 2 (11.5 %, 03 of 26). Out of these 26 mutation 23 were novel mutations and only 3 were previously reported. Table 2 is showing the deleterious mutations and levels of methylmalonic acid, C3, C3/C2 ratio, methionine and scores of Polyphen, SIFT and PROVEAN of each mutation in each patient.

Structural modelization shows that most of the mutations are located in C-terminal cofactor-binding domain (Fig. 3). Most of the missense mutations mapped on the MUT structure (except Ala631) were located far-off from the active site. The substitution of native amino acids induce steric clash in the surrounding area as a result disturbs the interactions with the neighbouring amino acid residues and thus affect the stability of the protein structure.

Discussion

To date near about 200 mutations have been identified in Methylmalonic acidemia patients that are reported in Human gene mutation database (www.hgmd.cf.ac.uk) [19]. A total of 70 mutations were identified in 21 patients. The mutation types were missense mutations (38/70, 54.2 %);

Fig. 2 Diagram of exon and intron showing various mutations and partial nucleotide alignment of the exon 12 of MCM for the studied patients, positions of individual mutations are indicated by arrow mark. Row 1 homo sapiens, row 2–13 studied patients and row 14 consensus sequence

frame shift mutations (16/70, 22.8 %); silent mutations (12/70, 17.1 %) and nonsense mutations (4/70, 5.7 %). Twenty-six (37.1 %) out of 70 mutations were predicted deleterious and 43 (61.4 %) were predicted benign. Most of these deleterious mutations were located in exon 12 (10/26, 38.4 %) followed by exon 9 (7/26, 26.9 %), exon 11 (6/26, 23.0 %) and exon 2 (3/26, 11.5 %).

A previous study stated that more than 75 % of the mut^o mutations belong to the barrel domain and missense mutations were the highest contributors for these mutations [8]. In this study we found 61.5 % (16/26) of these mutations belongs to the cobalamin binding domain (exon 11 and 12) while missense mutations are the highest contributors.

Another study found that 54 % mutations results in a premature termination of the amino acids [20]. We found only 38.4 % (10/26) mutations results in a premature

termination of the amino acids. In some previous studies they got large genomic deletions including entire exon 12 in the mut^o mutations [8, 12, 20, 21]. We didn't find any such large deletions; most of our mutations are point mutations or micro-rearrangements.

Acquaviva et al. stated that 67 % of the mutations are located in exons 2, 3, 11 and 12, which account for only 46 % of the coding sequences. They observed that non-sense mutations are restricted to the first eight exons. Worgan et al. [12] found that exons 2, 3, 6 and 11 harbored the most mutations. The spectrum or distribution of mutations in our study was much closer to the study done by Acquaviva as 73 % of the mutations were located in exons 2, 11, and 12 and only 26.9 % mutations were located in exon 9; however our study is restricted to four exons only. The distribution of the different types of mutations was not homogeneous in all exons.

Table 2 Depicts the deleterious mutations found and levels of methylmalonic acid, C3, C3/C2 ratio, methionine and scores of Polyphen, SIFT and PROVEAN of each mutation in each patient

ID	Age at diagnosis/gender	Presenting symptoms	Mutation 1 (Polyphen PROVEAN and SIFT score)	Mutation 2 (Polyphen PROVEAN and SIFT score)	Urinary MMA concentration	LCMSMS findings		
						C3 level	C3/ C2 ratio	Methionine
1	52 h/female	Lethargy, poor intake, respiratory distress, hyperammonemia and metabolic acidosis	p.V657E ^a 0.963 −3.59 0.000	p.Q660H ^a 0.572 −3.92 0.021	10,981.0	10.4	0.88	25.3
2	6 years/male	Global developmental delay, seizure, regression of milestones, hyperammonemia and metabolic acidosis	c.375_376insA (p.N126K ^a) 0.998 −4.36 0.003	p.K128I ^a 0.815 −2.88 0.000	9810.86	13.3	0.37	13.2
3	1 year/male	Global developmental delay, seizure	p.K621N Benoist et al. [22] 1.000 −4.90 0.000	p.G648D Crane and Ledley [26] 0.999 −6.50 0.000	5764.90	6.2	0.68	23.2
4	2½ months/male	Lethargy, poor intake, failure to thrive	c.1957delGA (p.T653L ^a) 0.998 −5.88 0.000	–	9305.00	8.2	0.76	12.3
5	9 months/female	Global developmental delay, hypotonia, failure to thrive	p.I647T ^a 0.858 −3.89 0.001	–	8688.70	8.2	0.78	15.6
6	4 months/female	Lethargy, poor intake, failure to thrive	c.1825_1826insT (p.E609V ^a) 0.025 −4.63 0.015	–	4280.94	8.0	0.56	22.1
7	1 year/male	Global developmental delay, seizure	p.E609* ^a	–	2807.12	8.9	0.65	27.3
8	6 months/male	Global developmental delay	p.K679N ^b 0.772 −3.50 0.021	–	7948.06	8.8	0.52	12.1
9	6 years/male	Vomiting, poor intake, regression of milestone	p.D554Y ^b 0.829 −4.09 0.001	p.E656D ^b 0.791 −2.91 0.001	2435.65	8.2	0.25	11.4
10	2 months/male	Lethargy, poor intake,	p.N6* ^a	–	3245.45	8.0	0.45	13.5
11	2 year/male	Global developmental delay, lethargy, hyperammonemia, metabolic acidosis	p.Q660H ^a 0.572 −3.92 0.021	p.I671* ^a	5006.65	8.1	0.56	17.3

Table 2 continued

ID	Age at diagnosis/gender	Presenting symptoms	Mutation 1 (Polyphen PROVEAN and SIFT score)	Mutation 2 (Polyphen PROVEAN and SIFT score)	Urinary MMA concentration	LCMSMS findings		
						C3 level	C3/ C2 ratio	Methionine
12	2 years/male	Global developmental delay, Abnormal body movements	c.1642delA (p.I548S ^a) 0.974 −3.50 0.000	p.D554Y ^b 0.829 −4.09 0.001	181,949.59	13.3	1.0	12.7
13	5 years/female	Global developmental delay	c.2062_2063insGA (p.E688 G ^b) 0.511 −4.10 0.025	−	3865.99	7.2	0.27	38.9
14	5 months/male	Seizure, lethargy, hyperammonemia	c.2014delA (p.S672A ^b) 1.000 −2.94 0.001	p.G696Y ^b 1.000 −7.49 0.000	7190.30	8.3	0.65	19.3
15	1 year/male	Global developmental delay,	p.A558T ^a 0.606 −2.14 0.038	−	5494.49	7.3	0.43	11.2
16	2 months/male	Vomiting, lethargy, poor intake	p.G539* ^a	−	3792.78	7.5	0.29	30.1
17	1 year/female	Poor intake, vomiting and dystonia	p.G630E Crane and Ledley [26] 1.000 −7.84 0.000	p. A631T 0.987 −3.32 0.002	7874.99	8.0	0.56	21.9
18	5 years/female	Regression of milestone, hearing and visual defects, dystonia, poor intake	c.1655delC (p.A552E ^a) 0.993 −4.07 0.000	p.R559P ^a 0.999 −6.86 0.000	15,599.0	9.9	0.56	12.6
19	1 year/female	Vomiting, dystonia, failure to thrive	p.N547T ^a 1.000 −5.88 0.000	−	13,061.1	8.8	0.89	22.1

^a Homozygous mutation, ^b Heterozygous mutation

Four novel nonsense mutations (Table 2) were detected in four patients. Three of which (p.G539*, p.E609* and p.I671*) are located downstream to exon 8. The insertion of thymine between nucleotide 15 and 16 in exon 2 introduces an early termination codon at position 6 (p.N6*) within the mitochondrial targeting sequences. Mutation in this part of exon produces an enzyme that does not reach to its target site as a result enzyme activity reduces. These mutations within the mitochondrial targeting sequence could produce truncated protein, which do not express

enzyme activity because the key portion of the amino terminus mature apoenzyme was lacking [3]. The substitution of nucleotide guanine to thymine at position 1615 in exon 9 introduces a termination codon at position 539 (p.G539*) within the linker sequence. This mutation also produces a truncated peptide that contain only the (β,α)8 barrel domain. Mutations (p.E609*) and (p.I671*) are located in the cofactor binding domain and may alter the correct binding of the cofactor. The mutation (p.E609*) is resulted due to insertion of thymine between nucleotide

Fig. 3 **a** Overall architecture of the enzyme methylmalonyl-CoA mutase with bound Cobalamin (Vitamin B12) in the catalytic centre and the structural locations of the observed missense mutations.

1824 and 1825 which introduces a termination codon at position 609. The mutation (p.I671*) is due to deletion of nucleotide adenosine at position 2011 that introduces stop codon at position 671 (p.I671*).

Seven novel frame shift mutations were detected in our patients. The frame shift mutation consist of two single nucleotide insertion (c.375_376insA and c.1825_1826insT), three single nucleotide deletions (c.1642delA, c.1655delC and c.2014delA), one insertion of two nucleotide (c.2062_2063insGA) and one deletion of two nucleotide (c.1957delGA). The three other frame shift mutations lead to introduction of termination codon: c.15_16insT in exon 2 (p.N6*), c.1824_1825insT in exon 11 (p.E609*) and c.2011delA in exon 12 (p.I671*).

Twelve novel missense mutations were found (p.K128I, p.N547T, p.D554Y, p.A558T, p.R559P, p.A631T, p.I647T, p.E656D, p.V657E, p.Q660H, p.K679N, and p.G696Y). These mutations alter the folding or structural stability of the protein as most of these are located in the secondary structure comprising of C terminal ($\beta\alpha$)₅ barrel (cobalamin binding domain). There were four mutations located in linker sequences encoded by exon 9. Mutation in this region can cause the absence of C terminal ($\beta\alpha$)₅ barrel (cobalamin binding domain) in the polypeptide chain.

b Native valine-657 substituted with longer and polar glutamate. **c** Native Arg-559 substituted with smaller and non-polar proline

Mutations p.K5N, p.R532L, p.E531D and p.M608L were predicted benign. These mutations are likely to be recurrent instead of hereditary. We found that various mutations occurred in close vicinity, in the same or nearby nucleotides, suggesting that these areas are hotspots for mutational events. In many cases this can be due to the presence of CpG dinucleotide, a mutational hotspot. The codon 532 was subjected to five different mutations- c.1524C>T (p.R532C), c.1525G>A (p.R532H), c.1524_1525delinAT (p.R532I), c.1524_1525delCG (p.R532L) and c.1524C>A (p.R532S). The codon 608 has also two mutations- c.1822A>C (p.M608L), c.1822_1823delinsTC (p.M608S) and codon 609 has three mutations- c.1825G>A (p.E609K), c.1825_1826insT (p.E609V) and c.1824_1825insT (p.E609*).

The sixteen mutations were located in sequences that code for the cofactor binding domain (one main structural domain) of the enzyme, with a heterogeneous distribution between exons 11 and 12. These mutations involve residues that lie at the surface of the cofactor crevice and may alter the contacts with the cofactor-binding domain or conformation of the crevice [22]. Most of the mutations are private mutations. There are many ethnic specific mutations documented in the published literature. The mutation p.G171V was a common mutation in black patients [5].

This mutation was present in 41 % of black patients. Likewise mutation p.R108C was seen in 60 % of Hispanic patients [12]. Two mutations p.G544X and p.G427D were present only in Asian patients [23]. The mutation p.N219Y was the first frequent mutation reported in Caucasian population [24], p.E117X in Japanese patients [6], c.671_678dup in Spanish patients [20] and c.1595G>A, c.2011A>G (p.I671V) in Filipino patients [25]. In our patients only one mutation c.2011A>G (p.I671V) which was specific for Filipino patients was present. This mutation was present in 52.3 % patients. It was difficult to correlate the clinical features with genotype, as most of the mutations were compound heterozygous and are novel.

One patient carrying mutations p.G630E and p.A631T in two consecutive amino acids in the polypeptide chain (Table 2). Substitution of glycine at position 630 results in the more severe mut^o phenotype [26]. This portion of protein consists of histidine loop. The histidine loop is a conserved structure that contains a consensus motif (DXHXXG) for cobalamin cofactor binding in enzymes [27]. These two mutations block the binding of adenosylcobalamin to methylmalonyl-CoA mutase by occluding the tail binding pocket.

The mutation p.G648D affects the histidine loop that leads to impaired function of cobalamin binding domain. Drennan et al. [27] stated that interactions between the histidine loop and the cobalamin are important both for catalysis and for binding of the cofactor. By molecular modeling they demonstrated that mutations involve the substitution of glycine within cobalamin binding domain by a large amino acid interferes sterically with the fit of the cobalamin tail into the B-fold of the cobalamin binding domain [27]. In mutation p.G696Y glycine has been substituted so it is likely that it also interferes with the fit of the cobalamin tail.

We have also observed some common polymorphism in our patients which were reported in literature. Three common polymorphism p.R532H [28], p.A664A [12] and p.I671V [29] were present in our patients. Substitution of guanine to adenosine at position 15 in exon 2 results into a silent mutation p.K5K [30]. This mutation was previously reported in Egyptian patient. This mutation was present in our one patient. The mutation p.K128K, located in the exon two is the most common mutation observed in our patients.

3D model of the human MCM enzyme and the structural locations of the observed missense mutations showing that most of them are located in C-terminal cofactor binding domain followed by interdomain linker sequence and N-terminal substrate binding domain.

In mutation p.V657E, mutant residue glutamate is longer as well as negatively charged as compared with native valine residue. Moreover the hydrophobic valine is buried and surrounded by other hydrophobic residues. The presence of a charged and longer glutamate in hydrophobic

area will push the surrounding residues apart. This substitution will induce steric clash in the surrounding area which is adjacent to the catalytic centre.

In mutation p.R599P, the substitution of arginine with smaller and non-polar proline will disturb polar interactions with the amino acid residues like Glu205 and Glu168 present in the neighbouring secondary structure and thus affect the stability of the protein structure. The broader proline is also making steric clashes with the neighbouring amino acid residues.

Conclusion

The spectrum of mutation included 38 missense mutations (54.2 %), 16 frameshift mutations (22.8 %), 12 silent mutations (17.1 %) and 4 nonsense mutations (5.7 %). All of them were point mutations or microrearrangements. Although the mutations were distributed throughout exons 2, 9, 11 and 12, 29.5 % of them were located in exon 9, 27.1 % in exon 12, 24.2 % in exon 2 and 18.5 % in exon 11. Nonsense mutations were distributed equally (1/4, 25 %) in each exon. In the same way, 34.2 % (13/38) of missense mutations were located in exon 9. Frame shift mutations were equally distributed in exon 2 and 9 (31.2 %, 5/16) followed by exon 12 (25.0 %, 4/16) and exon 11 (12.5 %, 2/16). Most of the mutations are private mutations while few occur recurrently. Mutations p.K5N, p.K128K, p.R532L, p.E531D and p.I671V were the most frequent mutations.

The impact of amino acid substitution on protein structure and function was predicted by analysis of multiple sequence alignments and protein 3-D structure. We found that most of the mutations that are deleterious to protein function and structure were located on exon 12 followed by exon 9, exon 11 and exon 2. In this study, nonsense mutations constituted 5.7 % of mutations, missense mutations constituted 17.1 % of mutations and 10 % of mutations are frameshift mutations. Although mutations are present in all four exons, most of the mutations that are deleterious to protein function were located on exon 12 (10 of 26, 38.4 %). Most of the mutations in our study were compound heterozygous as seen in methylmalonic acidemia. A total of 26 deleterious mutations identified. Three of them (p.K621N, p.G648D and p.G630E) are known mutations and 23 was novel mutations identified, contributing to existing database. We did not find any hotspot as the study subjects were less in number. To determine ethnic specific mutations in Indian patients further research is needed in a large number of patients.

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Compliance with Ethical Standards

Conflict of interest Authors declare that they have no conflict of interest.

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