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Identification of mutations, genotype–phenotype correlation and prenatal diagnosis of maple syrup urine disease in Indian patients

Deepti Gupta [a, b], Sunita Bijarnia-Mahay [a, *], Renu Saxena [a], Sudha Kohli [a], Ratna Dua-Puri [a], Jyotsna Verma [a], E. Thomas [a], Yosuke Shigematsu [c], Seiji Yamaguchi [d], Roumi Deb [b], Ishwar Chander Verma [a]

[a] Center of Medical Genetics, Sir Ganga Ram Hospital, Rajinder Nagar, New Delhi, India
[b] Amity Institute of Biotechnology, Amity University, Noida, U.P., India
[c] Department of Health Science, Faculty of Medical Sciences, University of Fukui, Matsuoka, Japan
[d] Department of Pediatrics, Shimane University School of Medicine, Izumo, Shimane, Japan

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ABSTRACT

Maple syrup urine disease (MSUD) is caused by mutations in genes *BCKDHA*, *BCKDHB*, *DBT* encoding E1α, E1β, and E2 subunits of enzyme complex, branched-chain alpha-ketoacid dehydrogenase (BCKDH). BCKDH participates in catabolism of branched-chain amino acids (BCAAs) – leucine, isoleucine and valine in the energy production pathway. Deficiency or defect in the enzyme complex causes accumulation of BCAAs and keto-acids leading to toxicity. Twenty-four patients with MSUD were enrolled in the study for molecular characterization and genotype–phenotype correlation. Molecular studies were carried out by sequencing of the 3 genes by Sanger method. Bioinformatics tools were employed to classify novel variations into pathogenic or benign. The predicted effects of novel changes on protein structure were elucidated by 3D modeling. Mutations were detected in 22 of 24 patients (11, 7 and 4 in *BCKDHB*, *BCKDHA* and *DBT* genes, respectively). Twenty mutations including 11 novel mutations were identified. Protein modeling in novel mutations showed alteration of structure and function of these subunits. Mutations, c.1065 delT (*BCKDHB* gene) and c.939G > C (*DBT* gene) were noted to be recurrent, identified in 6 of 22 alleles and 5 of 8 alleles, respectively. Two-third patients were of neonatal classical phenotype (16 of 24). *BCKDHB* gene mutations were present in 10 of these 16 patients. Prenatal diagnoses were performed in 4 families. Consanguinity was noted in 37.5% families. Although no obvious genotype–phenotype correlation could be found in our study, most cases with mutation in *BCKDHB* gene presented in neonatal period. Large number of novel mutations underlines the heterogeneity and distinctness of gene pool from India.

1. Introduction

Maple syrup urine disease (MSUD, OMIM 248600) is an autosomal recessive inherited metabolic disorder caused by deficiency of mitochondrial enzyme complex, branched-chain alpha-ketoacid dehydrogenase (BCKD) (Chuang and Shih, 2001). This enzyme complex is required for catalyzing the oxidative decarboxylation of branched chain keto-acids (BCKAs) that are derived from essential branched chain amino acids (BCAAs); leucine, isoleucine and valine. The metabolic block at this step results in the accumulation of BCAAs and their BCKAs in the body leading to severe symptoms of encephalopathy, seizures, developmental delay or even infantile death, if untreated (Chuang et al., 2006).

BCKD is a multimeric mitochondrial enzyme complex composed of four subunits namely E1α, E1β, E2, and E3, around a cubic core of 24 lipoate-bearing dihydrolipoyl transacylase (E2) subunit which binds the multiple subunits of BCKD decarboxylase (E1) and dihydrolipoamide dehydrogenase (E3) (Aevarsson et al., 2000). Two regulatory subunits; BCKD kinase and BCKD phosphatase are also attached to the complex. E1 is a thiamine dependent decarboxylase subunit, and is heteromeric, comprising of two α (E1α) and two β (E1β) subunits ($\alpha_2\beta_2$). Based on the genes involved, the types of MSUD are: – Type 1A (OMIM 608348) in case of mutations in *BCKDHA* gene encoding E1α subunit; Type 1B (OMIM 248611) for

* Corresponding author.
E-mail address: bijarnia@gmail.com (S. Bijarnia-Mahay).

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mutations in *BCKDHB* gene which encode E1β subunit and Type II (OMIM 248610) caused by mutations in *DBT* gene encoding E2 subunit. E3 component is common to BCKD, pyruvate dehydrogenase complex and alpha-ketoglutarate dehydrogenase complex and an impaired E3 activity results in combined ketoacid dehydrogenase deficiencies. Five types of clinical phenotypes based on age of onset, severity of clinical presentation, and response to thiamine have been reported (Chuang, 1998).

MSUD is rare in most populations with incidence of 1 in 150,000 live births in the general population and high incidence in some populations like the Mennonites (incidence is 1 in 176) (Danner and Doering, 1998). The incidence in India is currently not known in the absence of universal newborn screening program, but analysis of high risk cases show that it is one of the common metabolic disorders observed in clinical practice (Nagaraja et al., 2010).

We report here a clinical and genetic study which includes mutation analysis of genes, *BCKDHA*, *BCKDHB* and *DBT* in 24 unrelated Indian patients of MSUD.

2. Patient data

This study enrolled twenty-four Indian patients diagnosed with MSUD, referred for genetic testing at our center, from year 2010–2014. Spectrum of clinical symptoms, age of onset, family history of consanguinity and of any similar disease was noted. Parents were specifically asked for if there was a blood relationship between them that they were aware of. A four generation pedigree was used to rule out or rule in consanguinity. Biochemical testing (elevated BCAAs) on Tandem Mass Spectrometry (TMS) and/or abnormal metabolites (elevated BCKAs, 2-keto isocaproate, 3-hydroxy butyrate, 2-hydroxy isovalerate, 2-hydroxy caproate, 2-keto 3-methyl valerate) on a urinary organic acid profile using Gas Chromatography Mass Spectrometry (GC–MS) was performed to make the diagnosis. All biochemically proven cases were enrolled for molecular studies. Patients were categorized into two groups according to age of presentation as 'neonatal classic' or 'intermediate' presenting in infancy or later. A clinical follow up data was also gathered, wherever possible (Table 1).

3. Methods

For molecular study, blood samples were collected from patients after obtaining an informed consent from their parents. Since the probands were either young (less than 5 years of age) or intellectually disabled, informed consent was obtained from proband's parents. Samples from parents of 18 children were also collected.

3.1. Isolation of DNA and PCR

Genomic DNA was extracted using salt precipitation method (Miller et al., 1988). Coding regions as well as flanking exon–intron boundaries of genes (*BCKDHA*- 9 exons, *BCKDHB*- 10 exons, and *DBT*- 11 exons) were amplified by PCR. Primers for PCR were designed using web primer software (Supplementary Table 1 for the list of primer sequences and PCR amplification conditions).

3.2. Sequencing of PCR products

Amplified PCR products were checked on agarose gel and purified using multiscreen ®HTS Millipore vacuum manifold (Millipore, Massachusetts, USA). These purified products were then subjected to bidirectional sequencing on 3500 Genetic Analyzer (Applied Biosystems, Foster city, CA, USA). Sequences were analyzed by blasting them against the genomic sequences of *BCKDHA* (NM_000709.3), *BCKDHB* (NM_183050.2) and *DBT* (NM_001918.3) to determine all the mutations and variations.

3.3. Classification/annotation of novel variations

In-silico tools such as PolyPhen2 (Adzhubei et al., 2010), SIFT (Kumar et al., 2009), Mutation Taster (Schwarz et al., 2014), and MutPred (Li et al., 2009) software's were used to predict the effect of novel missense variations. The novel variations were also analyzed in 100 control alleles to rule out it being a polymorphism. These mutations were also checked in the 1000 genome project. Nonsense variations giving rise to truncated protein were considered pathogenic. Implication of splicing mutations was predicted using the BDGP site (Reese et al., 1997). I-mutant 2.0 based on the principle of free energy (DDG), was used to predict the stability/instability of the protein (Capriotti et al., 2005). Conservation of the aminoacid residues was checked using polyphen-2 and Mutation Taster software. Inheritance of mutations was confirmed in cases where parents samples were available.

3.4. Molecular modeling

Crystal structure with PDB ID – 1DTW was used as a template to perform computational modeling. The source organism for 1DTW is human. It has a resolution of 2.7 Å, obtained by X-ray diffraction method. The initial few amino acids of both subunits of BCKDH enzyme complex (1–45 for alpha chain, 1–50 for beta-chain) have not been crystallized. Therefore, the length of the crystallized alpha subunit (1DTW_A) is only 400 amino acids instead of 445 amino acids (46–445 amino acids of reference protein P12694), and that of beta subunit (1DTW_B) is 342 amino acids (51–392 amino acids for reference protein P21953) (Aevarsson et al., 2000). Swiss-Prot PDB Viewer software was used to visualize the effect of novel variations on protein structure (Guex and Peitsch, 1997).

4. Results

Of 24 patients enrolled in the study, 16 were males and 8 were females. Sixteen (63.6%) probands, presented in the neonatal period and were categorized into classical MSUD type. Remaining 8 (33.4%) patients presented in infancy (1month to 1year of age) or later, and were the intermediate type. Consanguinity was observed in 9 (37.5%) of 24 cases. Amongst these, 5 were from Muslim families and 3 were from South Indian Hindu community.

Main clinical features in the patients were seizures (in 17), acute encephalopathy (in 15), developmental delay (in 12), and failure to thrive (in 3). A family history of similar affection was present in 11(52.5%) of 21 families. Information was not available for 3 families.

A follow up data was obtained in 21 of 24 patients where information was available. Twelve (57.2%) patients were alive and on protein restricted diet, while the remaining nine (42.8%) died. Amongst surviving children, one is doing well after liver transplantation, 3 are in infancy with satisfactory outcome so far, and the other 8 patients have varying degrees of intellectual disability. Amongst deceased patients, majority died in early infancy, one child (in intermediate group) died at 5 years of age following a febrile illness (Table 1).

4.1. Molecular studies

Of 24 patients (48 alleles), mutations were identified in 43 (89.5%) alleles; 21 patients had mutations on both alleles and in one patient, mutation could be identified in only one allele. Mutation could not be identified in 2 neonates. Twenty different mutations were identified; 10 in *BCKDHB* gene, 8 in *BCKDHA* and 2 in *DBT* gene.