Meta Gene xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Meta Gene

# Molecular analysis of maple syrup urine disease in Jordanian families

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ARTICLE INFO

*Article history:*
Received 8 July 2015
Revised 27 August 2015
Accepted 21 October 2015
Available online xxxx

*Keywords:*
Maple syrup urine disease
Branched-chain α-ketoacid dehydrogenase complex
*BCKDHA*
*DBT*
Jordan

ABSTRACT

Maple syrup urine disease (MSUD) is an autosomal-recessive inborn error of amino acid metabolism characterized by the accumulation of three branched-chain amino acids (BCAAs) in patients' cells due to reduced activity of the branched-chain α-ketoacid dehydrogenase complex (BCKD). In this study, we aimed to sequence the coding exons of the *BCKDHA*, *BCKDHB*, and *DBT* genes in five Jordanian families with MSUD and one family from Iraq with MSUD. BCAA levels were measured in probands initially presenting with developmental delay and encephalopathy. All of the coding exons and flanking intronic sequences of the *BCKDHA*, *BCKDHB*, and *DBT* genes were amplified and subjected to direct DNA sequencing. Four different mutations in the *BCKDHA* gene were identified, including a novel frame-shift mutation, c.908_909delTG, in family 4. Two novel missense mutations at residues Met263 and Gly353 in the *DBT* gene were also found to be cosegregated with the MSUD phenotype in families 5 and 6, respectively. Structural analyses suggested that these two mutations may affect the assembly of the intermediate E2 trimer. No *BCKDHB* gene mutations were found in Jordanian patients. The mutation profiles described in this study provide a basis for the early detection of MSUD disease. To our knowledge, this is the first molecular study of MSUD in Jordan and Middle Eastern Arabic countries.



## 1. Introduction

Maple syrup urine disease (MSUD) is an autosomal recessive disease occurring in one of every 185,000 live births. MSUD is caused by a deficiency in the activity of any of the three catalytic subunits of the mitochondrial branched-chain α-ketoacid dehydrogenase (BCKD) complex (Chuang and Shih, 2001).

The mammalian BCKD complex is a 4-MDa mitochondrial multienzyme complex organized around a cubic core comprising 24 lipoate-bearing dihydrolipoyl transacylase (E2) subunits, to which multiple copies of branched-chain α-ketoacid decarboxylase (E1) and dihydrolipoamide dehydrogenase (E3) subunits are attached. In addition, two regulatory enzymes, a specific kinase and a specific phosphatase, are also a part of this complex (Chuang and Shih, 2001). In patients with MSUD, mutations in both *BCKDK* and *PPM1K* genes encoding mitochondrial BCKD kinase (BCKDK) and PP2Cm phosphatase, respectively, have been demonstrated, confirming the specific regulatory roles of these two proteins in modulating the catalytic activity of the BCKD complex (García-Cazorla et al., 2014; Oyarzabal et al., 2013).

The E2 subunit of the BCKD complex contains three independently folded domains: an N-terminal lipoyl-bearing domain (LBD), an internal subunit-binding domain (SBD), and a C-terminal inner core (catalytic) domain (CD), which are connected through flexible hinge regions that are rich in alanine, proline, and charged residues. The E2 subunits form trimeric entities, where eight trimers assemble into a cubic structure held together by trimer–trimer interactions, forming the core of the mammalian BCKD complex (Kato et al., 2006). Altogether, the BCKD complex is encoded by four nuclear genes: *BCKDHA*, *BCKDHB*, *DBT*, and *DLD*, which encode E1α, E1β, E2, and E3 subunits, respectively (Chuang and Shih, 2001). According to the public Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php/, accessed March 11, 2015), a total of 54 mutations in the *BCKDHA* gene (type Ia molecular subtype; MIM number: 608348), 52 mutations in the *BCKDHB* gene (type Ib; MIM number: 248611), and 46 mutations in the *DBT* gene (type II; MIM number: 248610) have been identified. Over 50% of the total number of mutations in the three genes are related to amino acid substitutions.

Clinically, MSUD is classified on basis of severity, the age at manifestation, residual BCKD activity, and thiamine responsiveness. Over 75% of the patients with MSUD are considered to have the classic form of the

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http://dx.doi.org/10.1016/j.mgene.2015.10.002

disease, where their residual BCKD activity is less than 2% of the control enzyme activity. These patients often exhibit encephalopathy and coma during neonatal life as a result of accumulation of valine, leucine, and isoleucine, the three branched-chain amino acids (BCAAs) found in cells and body fluids (Chuang and Shih, 2001).

To our knowledge, this study is the first to report the molecular basis of MSUD in Jordanian families.

## 2. Materials and methods

### 2.1. Families

This study was approved by the Institutional Review Board of the Jordan University of Science and Technology and King Abdullah University Hospital. Consent was obtained from the patients' parents. All patients with the exception of one (proband 2) were the offspring of consanguineous parents. DNA samples were collected for 50 control individuals, from all patients, parents, and siblings of families 2, 4, 5, and 6 (Table 1). The DNA samples were used for screening for mutations in the *BCKDHA*, *BCKDHB*, and *DBT* genes (Table 1).

### 2.2. Mutation analysis

Genomic DNA from patients and their available family members was extracted from whole blood using a PureGene Blood Core Kit B (Qiagen, Hilden, Germany). Polymerase chain reaction (PCR) was performed in a Veriti thermal cycler (Applied Biosystems, Foster City, CA, USA). The forward and reverse PCR primers for amplifying *BCKDHA* exons 1–9, *BCKDHB* exons 1–11, *DBT* exons 1–11, and their flanking intronic sequences were designed using the Primer3 algorithm (http://frodo.wi.mit.edu/primer3) according to the following GenBank cDNA and genomic DNA accession numbers: NM_000709.3 and NG_013004.1 for the *BCKDHA* gene, NM_000056.3 and NG_009775.1 for the *BCKDHB* gene, and NM_001918.3 and NG_011852.2 for the *DBT* gene. All primer sequences and PCR reaction conditions are available on request.

The purified PCR products were subjected to bidirectional sequencing using the Big-Dye Terminator v3.1 Cycle Sequencing Kit and applied to a 3130xl Genetic Analyzer (Applied Biosystems). The sequence data were compared to the normal *BCKDHA*, *BCKDHB*, and *DBT* gene sequences using the ChromasPro 1.34 (Technelysium Pty Ltd., Australia) software package. Sequence nomenclatures for the coding and non-coding variants are described in accordance with the Human Genome Variation Society Nomenclature standards (http://www.hgvs.org/mutnomen), where +1 is the A of the ATG translation initiation codon for each cDNA of the three genes. The residues are numbered according to P12694 (E1α), P21953 (E1β), and P11182 (E2) UniProtKB accession numbers, where the mitochondrial signal peptides are counted.

The functional impact of the three missense variants of the E2 protein was predicted by PolyPhen2 software (http://genetics.bwh.harvard.edu/pph2/).

### 2.3. Amino acid quantification

Amino acid profiles were determined using the 6 N HCl hydrolysis method based on ion exchange chromatography followed by post-column derivatization with ninhydrin using an S433 Amino-Acid Analyzer (SykamGmbH, Eresing, Germany).

### 2.4. 2.5 Phylogenetic conservation and structural analyses of the E2 missense mutations

Multiple E2 amino acid sequence alignments were generated using Clustal Omega. Pathogenicity prediction of the Met263 and Gly353 mutations was performed using the PolyPhen program (http://genetics.bwh.harvard.edu/pph/). Structural analyses were carried out based on the crystal structure of bovine E2 (PDB: 2IHW) using PYMOL (http://www.pymol.org).

## 3. Results

All probands, with the exception of number 5 (who was on a low-protein diet), exhibited high plasma BCAA levels at the time of referral to the Metabolic Genetics Clinic at King Hussein Medical Center (Table 1). The symptom severity and age of onset of the patients when they encountered their first metabolic crises varied (Table 1). Initially, we sequenced the nine exons and flanking intronic regions of the *BCKDHA* gene for all six probands. For patients not harboring mutation(s) in this gene, DNA samples were sequenced to determine whether there were mutations in the 11 exons of each of the *BCKDHB* and *DBT* genes.

### 3.1. BCKDHA gene mutations

We identified two frame-shift mutations, one splicing defect, and one missense mutation in the *BCKDHA* gene in four out of six families included in this study. The first frame-shift mutation was a single base deletion in exon 9 in the proband of family 1. The c.1251delC p.Aal418Profs*67 mutation (Fig. 1A) resulted in disruption of the physiological stop codon of the gene. Consequently, the C-terminal of the E1α subunit was extended by an additional 38 residues before a new downstream stop codon was introduced.

**Table 1**
Clinical and laboratory findings of Jordanian patients with MSUD.

| Patient | Sex | Age | Age at diagnosis | Clinical presentation | Leucine μmol/L[a] | Isoleucine μmol/L[a] | Valine μmol/L[a] |
|---|---|---|---|---|---|---|---|
| 1 | M | 5 years and 5 months | 20 days | Acute encephalopathy and intractable seizures at presentation, later follow up psychomotor retardation, axial hypotonia, brain atrophy | 1928 | 411 | 509 |
| 2[b] | F | 3 years | 6 months | Chronic encephalopathy, spasticity, moderate developmental delay | 951 | 148 | 350 |
| 3[c] | M | 1 year[c] | 7 days | Hypoactivity and acute encephalopathy at presentation, later follow up severe developmental delay, and brain atrophy | 2794 | 419 | 859 |
| 4[d] | M | 5 years and 6 months | 18 months | Mild developmental delay, infrequent partial seizures | ND[e] | ND | ND |
| 5[f] | F | 5 years and 5 months | 3 years | Moderate developmental delay, hypotonia, mild brain atrophy | 165 | 96 | 193 |
| 6[f] | F | 8 years | 3 days | Poor feeding and convulsions at presentation, later follow up moderate developmental delay | 1083 | 241 | 573 |

[a] Normal ranges are as follows: leucine, 47–155 μmol/L; isoleucine, 31–86 μmol/L; and valine, 64–294 μmol/L.
[b] Had two normal sisters and one affected cousins from consanguineous marriages.
[c] Patient died at this age.
[d] Iraqi national, had one affected sibling.
[e] ND, no data.
[f] Had a sibling who died of MSUD.

Please cite this article as: Jaradat, S.A., et al., Molecular analysis of maple syrup urine disease in Jordanian families, Meta Gene (2015), http://dx.doi.org/10.1016/j.mgene.2015.10.002