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Short Communication

Molecular characterization of maple syrup urine disease patients from Tunisia

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ABSTRACT

Maple syrup urine disease (MSUD) is a rare disorder of branched-chain amino acids (BCAA) metabolism caused by the defective function of branched-chain α-ketoacid dehydrogenase complex (BCKD). The disease causal mutations can occur either in *BCKDHA*, *BCKDHB* or *DBT* genes encoding respectively the E1α, E1β and E2 subunits of the complex. In this study we report the molecular characterization of 3 Tunisian patients with the classic form of MSUD. Two novel putative mutations have been identified: the alteration c.716A>G (p.Glu239Gly) in *BCKDHB* and a small deletion (c.1333_1336delAATG; p.Asn445X) detected in *DBT* gene.

1. Introduction

Maple syrup urine disease (MSUD; OMIM ID #248600) is a rare autosomal recessive disorder of metabolism caused by the defective function of the branched-chain α-ketoacid dehydrogenase complex (BCKD; EC 1.2.4.4) (Chuang and Shih, 2001).

BCKD catalyzes the oxidative decarboxylation of branched-chain α-ketoacids (BCKAs) derived from the transamination of branched-chain amino acids (BCAAs) leucine, isoleucine and valine. This mitochondrial complex is composed by three catalytic components including a branched-chain α-ketoacid decarboxylase (E1), formed by two E1α and two E1β subunits, a dihydrolipoyl transacylase (E2) and a dihydrolipoamide dehydrogenase (E3). All the components are encoded by nuclear genes: *BCKDHA* and *BCKDHB* encode E1α and β, respectively; *DBT* codes for E2, and *DLD* for E3. Two regulatory enzymes, a branched-chain ketoacid dehydrogenase kinase (*BCKDK*) and a branched-chain ketoacid dehydrogenase phosphatase, protein phosphatase 2Cm (*PPM1K* or *PP2Cm*) are also part of the complex (H. et al., 1978; Lu et al., 2009; Reed et al., 1985).

When BCKD activity is impaired both BCAAs and the respective BCKAs accumulate in the body tissues and fluids reaching toxic levels which cause severe clinical consequences primarily triggered by leucine (leucinosis) (Morton et al., 2002). High heterogeneity in phenotypic presentation of MSUD is reflected on the five forms of the disease: classic, intermediate, intermittent, thiamine responsive and E3 deficient. The majority of affected individuals (~75%) has the classic form of the disease which is also the most severe with neonatal presentation (Chuang and Shih, 2001).

At the molecular level, the mutational spectrum of MSUD encompasses a wide number of pathogenic alterations either in *BCKDHA*, *BCKDHB* or *DBT*, with each gene accounting for approximately identical amount of mutations (Nellis and Danner, 2001). However, no phenotype–genotype correlation could be established.

Nowadays Tunisia faces the challenge of an epidemiological transition, with the increase in the prevalence of non-communicable diseases (Romdhane et al., 2011). Furthermore, one of the main demographic features of the Tunisian population is the high rate of consanguinity, a long lasting tradition throughout the entire Arab World that greatly accounts for the very high prevalence of genetic diseases in some Arab populations (Romdhane et al., 2011). In Tunisia, no information was available on the incidence and epidemiology of genetic disorders until this year, when Hadj-Taieb et al., based on a retrospective study, reported data on inborn errors of metabolism in the country (Hadj-Taieb et al., 2012). One of the most common disorders of amino acid metabolism was MSUD, reaching the incidence of 1 in 13,716 live births (adjusted for the level of inbreeding in the population), which is notably higher than the estimate of 1 in 185,000 infants worldwide (Chuang and Shih, 2001). Since MSUD might present a major cause of mortality, disability, and chronic disease in Tunisia, it

Abbreviations: MSUD, maple syrup urine disease; BCAA, branched chain amino acids; BCKD, branched chain a ketoacid dehydrogenase complex; *BCKDK*, branched chain ketoacid dehydrogenase kinase; *PPM1K*/*PP2Cm*, protein phosphatase 2Cm.

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seemed important to explore the mutational bases of the disease due to the implications of the knowledge in terms of healthcare.

In the present study we have analyzed DNA samples of three classic MSUD patients from Tunisia. Two new putative causal mutations have been identified: one in *BCKDHB* (c.716A>G; p.Glu239Gly) and the other in *DBT* (c.1333_1336delAATG; p.Asn445X). As the best of our knowledge, this constitutes the first work on the mutational analysis in MSUD Tunisian patients, whose findings will allow providing a successful prenatal diagnosis to their at-risk families.

2. Materials and methods

2.1. Subjects

The cases presented in this study refer to a male and female child, from the same city in the center of Tunisia, Sidi Bouzid, and a male child from Sousse, which is in the central-east of the country. All patients had a clinical diagnosis of classic MSUD that was confirmed by elevated levels of plasma branched-chain amino acids together with the presence of L-alloisoleucine. Informed consent was obtained from patient's parents.

2.1.1. Patient 1

Female infant, 2-days-old when MSUD was suspected. Although appearing normal at birth, in a few days she presented lethargy, irritability and poor feeding, developed dystonia and alternating hypotonia and hypertonia accompanied by ketoacidosis and a fenugreek odor of the urine (Monastiri et al., 1997). The clinical diagnosis could only be confirmed later with the plasma amino acid analysis. Elevated BCAAs have been observed, with leucine at 4443 μmol/L (normal range 55–167 μmol/L), isoleucine at 521 μmol/L (normal range 52–283 μmol/L) and valine at 829 μmol/L (normal range 34–261 μmol/L); level of alloisoleucine was 326 μmol/L. She was diagnosed with the classic form of MSUD and had a low protein diet but died 30 days after birth. Her parents are first cousins.

2.1.2. Patient 2

Young male, 3-days-old when the first signs of MSUD were observed: refusal to drink, poor feeding, irritability, seizures, lethargy, hypotonia, hypertonia and ketoacidosis. The clinical diagnosis was confirmed by urine and conventional blood analysis, with leucine at 4290 μmol/L, isoleucine at 748 μmol/L, valine at 921 μmol/L and alloisoleucine at 320 μmol/L. He received a low protein diet and special milk to reduce BCAAs intake and is currently 16 years old but is mentally retarded. His parents are first cousins.

2.1.3. Patient 3

Male infant, 6-days-old when the severe form of MSUD was suspected through the clinical signs developed: abnormal movements, refusal to drink, poor feeding, irritability, convulsions, spasticity, lethargy, ketoacidosis and also the specific odor of urine. He had a dietary protein restriction but died a month later. His parents are first cousins.

2.2. Mutation analysis

Peripheral blood samples from patients and parents were obtained by venipuncture in EDTA tubes. The DNA was extracted using the salting-out technique (Miller et al., 1988).

The entire coding and flanking intronic regions of *BCKDHA*, *BCKDHB* and *DBT* genes were amplified from genomic DNA using standard protocols. Direct sequencing, with forward and reverse primers, was performed on an Applied Biosystems 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The presence of the mutations was confirmed by independent amplification and sequencing reactions. Primer sequences and PCR conditions are available under request and have been used previously (Quental et al., 2008).

The sequences have been compared with reference sequences with accession numbers: *BCKDHA* NM_000709.3; *BCKDHB* NM_000056.3 and *DBT* NM_001918.2. Mutation nomenclature follows the recommendations of Human Genome Variation Society (http://www.hgvs.org/mutnomen/), with nucleotide +1 corresponding to the A of the ATG translation initiation codon (den Dunnen and Antonarakis, 2000).

PolyPhen2 software was used to predict the functional effect of the missense alteration to the protein (http://genetics.bwh.harvard.edu/pph2/) (Adzhubei et al., 2010).

The E1β protein structural model with the mutation was built with MODELLER (Eswar et al., 2002), using the normal human sequence (PDB ID: 1X7Y) as template (Wynn et al., 2004); generation of protein plots was performed using PyMOL (http://www.pymol.org) (DeLano).

The 1000 Genomes Project (http://www.1000genomes.org/) data, comprising a total of 2184 chromosomes from different ethnic backgrounds, including 494 of African ancestry as well as the Exome Variant Server database (http://evs.gs.washington.edu/EVS/) (Nov 2012) were used to test if the newly identified alterations were present in control individuals (Altshuler et al., 2010).

3. Results and discussion

The aim of this study was to perform the molecular characterization of three Tunisian patients with maple syrup urine disease.

The analysis of the entire coding region of *BCKDHA*, *BCKDHB* and *DBT* genes allowed the successful identification of the putative disease causal mutations in all the patients. Two of them (patients 1 and 2) share the same homozygous molecular alteration in *BCKDHB* gene. The nucleotide change is c.716A>G, which causes the replacement of a glutamic acid by a glycine (p.Glu239Gly); this mutation has not been previously identified. Samples from parents of patient 2 were available and both were found to be carriers of the alteration.

In order to evaluate whether the alteration could be a rare polymorphic nucleotide change, we have analyzed sequences from 2184 chromosomes (494 of them of African ancestry) available from the 1000 Genomes Project, which lead to conclude that the alteration was not present in any of them. The same result was observed in the data from Exome variant server, therefore reinforcing the pathogenic potential of the variation identified.

Then, we assessed the possible functional impact of the nonsynonymous substitution (p.Glu239Gly) using PolyPhen2, that predicted it to be “probably damaging” (HumVar score of 0.999).

To have further insights on the functional effect caused by the p.Glu239Gly replacement, 3D analysis (Fig. 1) of the wild-type and mutant proteins was conducted. Glu239-β is involved in side-chain interactions between β-strand g and the parallel β-strand e, through the establishment of a salt bridge with residue Arg183-β (Fig. 1B); this interaction must be extremely important for the maintenance of the proper five stranded parallel β-sheet conformation. In the modeled protein structure harboring the Gly239-β residue (Fig. 1C) the H-bonded salt bridge is lost, which probably will disturb the referred β-sheet folding pattern of the protein and consequently its function. Moreover, residue Arg183-β is in close proximity to the binding site 2 of K^+ ion, thus being predictable that any structural alteration in this region might affect ion binding that is critical for the stability and activity of the complex (Aevarsson et al., 2000; Edelmann et al., 2001; Wynn et al., 2001). Also expectedly, this alteration in the K^+ ion-binding pocket will disrupt the interaction between the α and β subunits, and thus the E1 tetrameric integrity, since residue Asn233-β that binds K^+ directly interacts with residues of the α subunit (Gln414-α and Glu415-α) (Aevarsson et al., 2000).

In the modeled 3D structure harboring the mutation, it was possible to observe that additional structural changes which do not directly involve the mutated residue occur to accommodate the glycine at position 239 (Figs. 1B and C). These alterations include loss of the H-bond salt bridges that are established in the wild-type protein