Molecular genetics of maple syrup urine disease in the Turkish population

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In maple syrup urine disease (MSUD), disease-causing mutations can affect the *BCKDHA*, *BCKDHB* or *DBT* genes encoding for the E1α, E1β and E2 subunits of the multienzyme branched-chain α-keto acid dehydrogenase (BCKDH) complex. Here we summarize the MSUD genotypes of a cohort of 32 unrelated Turkish patients in whom both alleles at a single gene locus harbored presumable disease-causing nucleotide changes. The patients had different forms of MSUD, ranging from the severe classical form (26 patients) to severe and mild variants (6 patients). In all except two patients (92%), the mutations occurred homozygously. The mutational spectrum included 27 different sequence variations - 12 changes in the *BCKDHA*, 10 in the *BCKDHB*, and 5 in the *DBT* genes. In 37% (12 patients) of a total of 64 alleles, the supposed disease-causing mutations were located in the *BCKDHA* gene, in 44% (14 patients) in the *BCKDHB* gene and in 19% (6 patients) in the *DBT* gene. The mutational profile is heterogeneous, although two mutations occurred three times and five mutations occurred twice. There was no cluster for a single mutation except for c.773G>A (p.Cys258Tyr) in the *BCKDHA* gene, a hypothetical founder mutation in the Çamlıdere population.

Key words: maple syrup urine disease, branched-chain α-keto acid dehydrogenase complex, BCKDHA, BCKDHB, DBT.

Maple syrup urine disease (MSUD, McKusick 248600) is a rare autosomal recessive inborn error of metabolism that causes acute and chronic brain dysfunction. In populations with a high rate of consanguineous marriages, such as in Turkey, the incidence is notably high and may be as high as 1 in 50,000 newborns. MSUD seems to be the most frequently occurring organic acidemia in Turkey[1].

Maple syrup urine disease is caused by a defective activity of the branched-chain α-keto acid dehydrogenase (BCKDH) complex. Due to the inherited metabolic block, the branched-chain amino acids leucine, valine and isoleucine and the corresponding branched-chain α-keto acids accumulate. The BCKDH is a multienzyme complex composed of a multimeric dihydrolipoamide transacylase (E2) core to which multiple copies of BCKDH decarboxylase (E1) and dihydrolipoamide dehydrogenase (E3) as well as two regulatory enzymes, BCKDH kinase and BCKDH phosphatase, are bound[2,3]. The E1 component exists as a heterotetramer composed of two E1α and two E1β subunits. The genomic changes that impair BCKDH activity can occur in any of the catalytic components of the complex, but both alleles at a single gene locus must harbor nucleotide changes[4-7]. Based on the affected loci of the BCKDH complex, three molecular MSUD genotypes are known thus far: subtype Ia for mutations affecting the E1α (*BCKDHA*) gene, subtype Ib for mutations affecting the E1β (*BCKDHB*) gene and subtype II for mutations affecting the E2 (*DBT*) gene.

About 75% of MSUD patients have the severe classic form (<2% of control enzyme activity) with neonatal onset of encephalopathy and coma. About 25% of patients suffer from variant forms (with a continuum of residual BCKDH activity from 2 to 40%) with later onset or absence of cerebral symptoms[4]. Based on the clinical presentation and biochemical response to thiamine administration, variants can be divided into more severe so-called intermediate (in the present paper called severe variants), milder so-called intermittent (in the present paper called mild variants), and thiamine-responsive forms[4] as well as an asymptomatic phenotype that can be identified by newborn screening[8].

In the present study, we analyzed DNA samples of 19 Turkish patients with MSUD for mutations in the *BCKDHA*, *BCKDHB*, and *DBT* genes of the BCKDH complex. In addition, we summarize the newly investigated and the thus far communicated genotypes, for a total cohort of 32 unrelated Turkish patients with different forms of MSUD.

Material and Methods

In addition to the thus-far communicated genotypes of 16 unrelated patients of Turkish origin with different forms of MSUD[1,9-11], we performed mutation analyses in another 19 Turkish MSUD patients. For that, the exonic coding sequences of all three genes (*BCKDHA* with 9, *BCKDHB* and *DBT* with 11 exons each) derived from peripheral blood leukocytes were studied from the patients and their parents. Three patients were migrants living in Germany and Austria. From 16 patients and their parents, DNA samples were prepared in İstanbul and Ankara and sent for analysis to Düsseldorf. All patients except two had a consanguineous background. All families were asked for their origin in Turkey.

Informed consent for the analyses was obtained from a parent/legal guardian of the patients. The Heinrich-Heine University Institutional Review Board approved the study. For assessment of the pathogenicity of the novel mutations, 50 *BCKDHA*, 45 *BCKDHB* and 50 *DBT* control alleles of a Turkish population were studied.

Genomic DNA was extracted from peripheral blood leukocytes. Mutation analysis was performed by direct sequencing of polymerase chain reaction (PCR) fragments obtained after amplification of the exonic and flanking intron region coding sequences of the three (*BCKDHA*, *BCKDHB*, *DBT*) genes. Primers to amplify the genomic DNA samples were designed according to GeneBank sequences. All primer sequences are available on request. Direct cycle sequencing of all PCR fragments was performed with BigDye Terminator v 3.1 mix (Applied Biosystem; Foster City, CA, USA) and analyzed by capillary electrophoresis on an ABI prism 310 Genetic Analyzer (Applied Biosystems).

Analyzed sequences were compared with the cDNA and genomic DNA sequences in GenBank accession numbers NM_000709 (*BCKDHA* gene, contig NT_011109), NM_000056 (*BCKDHB* gene, contig NT_007299) and NM_001918 (*DBT* gene, contig NT_028050). The mutation nomenclature used follows the recommendation of the Human Genome Variation Society (http://www.hgvs.org/mutnomen). cDNA numbering commences from the ATG start codon, where +1 is the A of the ATG translation initiation codon.

Results

Genotypes of 19 Newly Investigated MSUD Patients

In the present study, we have analyzed the entire coding region of the *BCKDHA*, *BCKDHB*, and *DBT* genes in a cohort of 19 MSUD patients. In 16 of them (14 classical and 2 severe variants), molecular characterization was successfully completed by identifying nucleotide changes in both alleles at a single gene locus. In one patient, only one mutation could be detected and in two patients no mutation was found. In total, we have identified 16 different nucleotide sequence variations that presumably lead to loss-of-function of the BCKDH complex. Distribution of the mutations between the three genes was as follows: 5 affected the *BCKDHA*, 8 the *BCKDHB* and 3 the *DBT* genes. Two mutations have already been reported in the literature, 14 were novel. In all except two patients, the mutations occurred homozygously. It was possible to extend the DNA molecular characterization to the parents of 15 patients. In all cases, they were found to be carriers of the mutation detected in their offspring.

Mutations in the *BCKDHA* gene. Five different nucleotide changes were identified in the *BCKDHA* gene. All were novel. Two point

mutations (c.783G>A and c.784C>A) were lying side by side, together giving rise to a stop in codon 258 of the amino acid sequence (p.Cys258Stop). The other nucleotide changes comprised a nonsense mutation c.205C>T (p.Gln69Stop) and two splice site mutations, IVS6-1G>C and IVS8-2A>G.

Mutations in the *BCKDHB* gene. Eight different mutations were detected in the BCKDHB gene. Two nonsense mutations, c.853C>T (p.Arg285Stop) and c.1149T>A (p.Tyr383Stop), were already known from the literature, whereas three nonsense mutations, c.331C>T (p.Arg111Stop), c.564T>A (p.Cys188Stop) and c.688G>T (p.Glu230Stop), are described here for the first time. The three missense mutations, c.272C>T (p.Ala91Val), c.547C>T (p.Arg183Trp) and c.1015T>C (p.Ser339Leu), were novel mutations.

Mutations in the DBT gene. Two novel missense mutations, c.788T>G (p.Met263Arg) and c.1202T>C (p.Ile401Thr), and one novel splice site mutation, IVS 8-1G>A, were identified in the DBT gene.

Compilation of the Molecular Genetic Data of Communicated Turkish MSUD Patients

In Table I, the genotypes of a cohort of 32 unrelated Turkish patients in whom disease-causing mutations are known in both alleles at a single gene locus are summarized. In all except two patients (92%), the mutations occurred homozygously, corresponding to the evidence of the patients' consanguineous background. In 37% (12 patients) of a total of 64 alleles, the supposed disease-causing mutations were located in the *BCKDHA*, in 44% (14 patients) in the *BCKDHB*, and in 19% (6 patients) in the DBT genes. In the *BCKDHA* and *BCKDHB* genes, all allelic variants were nucleotide substitutions. In the *DBT* gene, the allelic variants were nucleotide substitutions and one deletion. In addition to the disease-causing mutations, various patients had different nucleotide sequence variations in the *BCKDHA* gene, such as c.87C>A (p.Pro29His) - registered as SNP rs 34589432, c.34C>A (p.Arg12Arg) - registered as SNP rs 34541442, c.972C>T (p.Phe324Phe) - registered as SNP rs 284652, c.1222A>G (p. Leu407Leu) - registered as SNP rs 4647, the nucleotide sequence variation in the DBT gene, c.1150G>A (p.Gly384Ser) - registered as SNP rs 12021720, and different intronic polymorphisms.

Discussion

Genotypes of 19 Newly Investigated MSUD Patients

Pathogenicity of the novel mutations was assessed by discarding their presence in 50 *BCKDHA*, 45 *BCKDHB* and 50 *DBT* control alleles of Turkish individuals. None of the novel mutations was registered as a non-synonymous coding single nucleotide polymorphism. The disease-causing effect was assumed when the alteration led to a premature termination codon and when splicing mutations were located in the consensus sequence of the acceptor site of the genes. For the novel missense variations, all affected residues were located in the essential secondary structure elements' strands and helices. The novel missense variations affected highly conserved residues between the human E1 or E2 component and their homologous proteins as compared in the nucleotide database from bacterial (*Pseudomonas putida*) and animal (Bos taurus, Rattus norvegicus, Gallus gallus) genomes[6,12], strengthening their impact on the structure/function of the proteins.

In the *BCKDHB* gene, three novel missense mutations were identified, leading to the amino acid changes p.Ala91Val, p.Arg183Trp, and p.Ser339Leu. They can be discussed on the basis of the effect on the BCKDH (E1) structure[6]. With respect to the p.Ala91Val change, an extreme structural impairment and loss of function are scarcely expected, since the nonpolar alanine, which is located in strand b of the β subunit, is substituted by the nonpolar and only slightly bigger valine. This is in fair agreement with the clinical data, since patients 14 and 26, homozygous for p.Ala91Val, presented a variant form of MSUD, having been diagnosed at four months and two years of age, respectively. Another newly detected mutation within E1β was p.Arg183Trp. Here the residue Arg183 is located in strand e in the immediate neighborhood of the binding site 1, involved in the structurally important K^+ binding and being also important for subunit association. The substitution of the positively charged arginine by the bulky aromatic and uncharged tryptophan in this critical region would clearly have a strong negative effect on the structural integrity and function of the protein. With respect to p.Ser339Leu, the third newly detected missense variation within E1β, the

Table I. Mutations Detected in the BCKDHA, BCKDHB and DBT Genes in the Turkish Population

					Mutation			
						Sequence		
	Gene	Patient	Phenotype	Age at diagnosis	Exon	Nucleotide	Protein	Reference of published mutations
BACKDHA (E1α)		11	Classical	9 days	2	c.205C>T (het)	Gln69Stop	Novel
					6	c.783G>A (het) &		Novel
					6	c.784C>A (het)	Cys258Stop	Novel
		D[#1]	Classical	6 days	3	c.373C>G (hom)	Gln125Glu (Q80E)	1
		25	Classical	11 days	IVS6	IVS6-1G>C (hom)	Splicing	Novel
		5[8,11]	Severe variant	5 years	6	c.757G>A (hom)	Ala253Thr (A209T)	14
		A,B,C[1]	Classical	7 days	6	c773G>A (hom)	Cys258Tyr (C213Y)	1
		4[9]	Classical	8 days	7	c859C>T (hom)	Arg287Stop (R242X)	15
		18[9]	Severe variant	21 days	7	c.868G>A (hom)	Gly290Arg (G245R)	16
		16[8,11]	Mild variant	6 days	7	c.919G>A (hom)	Arg297His (R252H)	4
		17[8,11]	Mild variant	2 months	7	c.982G>A (hom)	Ala328Thr	11
		33	Classical	14 days	IVS8	IVS8-2A>G (hom)	Splicing	Novel
BACKDHB (E1β)		14	Severe variant	4 months	2	c.272C>T (hom)	Ala91Val	Novel
		26	Severe variant	2 years	2	c.272C>T (hom)	Ala91Val	Novel
		24	Classical	12 days	3	c.331C>T (hom)	Arg111Stop	Novel
		34	Classical	10 days	5	c.547C>T (hom)	Arg183Trp	Novel
		10	Classical	5 days	5	c.564T>A (hom)	Cys188Stop	Novel
		15[9,10]	Classical	11 days	6	c.665A>G (hom)	Lys222Arg	9
		1	Classical	3 days	6	c.688G>T (hom)	Glu230Stop	Novel
		21[#9,10]	Classical	17 days	7	c.752T>C (hom)	Val251Ala	5
		29[9,10]	Classical	11 days	8	c.853C>T (hom)	Arg285Stop	9
		7	Classical	1 day	8	c.853C>T (hom)	Arg285Stop	9
		3	Classical	18 days	9	c.1015T>C (hom)	Ser339Leu	Novel
		19[9,10]	Classical	14 days	10	c.1149T>A (hom)	Tyr383Stop	5
		22[9,10]	Classical	15 days	10	c.1149T>A (hom)	Tyr383Stop	5
		23	Classical	7 days	10	c.1149T>A (hom)	Tyr383Stop	5
DBT (E2)		E[1]	Classical	10 days	IVS3	IVS3-1G>A (hom)	Splicing	1
		8	Classical	14 days	7	c.788T>G (hom)	Met263Arg	Novel
		32	Classical	14 days	7	c.788T>G (hom)	Met263Arg	Novel
		12[9,10]	Classical	13 days	8 (7)	c.940-1G>A (hom)	Ala314-Lys339del	9
		27	Classical	7 days	IVS8	IVS8-1G>A (het)	Splicing	Novel
					9	c.1202T>C (het)	İle401Thr	Novel
		31	Classical	12 days	9	c.1202T>C (hom)	İle401Thr	Novel

Patient column: The reference numbers relate to one or two previous publications in which the patient was already reported.
[#] Patients carry the disease-causing mutation c.452C>T in one BCKDHA allele (p.Thr151Met, previously named T106M[1]).
Phenotype column: For definition of severe and mild variants, see Introduction.
Mutation columns: (Hom) and (het) are abbreviations for homozygous and heterozygous.
Mutations at protein level in brackets: old nomenclature.
In one newly investigated patient only one p.Arg363Trp mutation in the BCKHA gene was found.

Ser339 residue w located in helix 11 and a region important for the β'β subunit interaction. Serine with an uncharged polar side chain is substituted by nonpolar leucine and might impair the β'β assembly interfaces and subunit interaction. Both mutations, p.Arg183Trp and p.Ser339Leu, in a homozygous fashion are associated with a severe neonatal course of MSUD.

Two novel missense mutations, p.Met263Arg and p.Ile401Thr, were found in the DBT gene. They affect residues located at the E2 inner core (catalytic) domain (CD); the crystal structure of its human form has not yet been determined[13]. At position 263, where patients 8 and 32 had an arginine substitution in a homozygous fashion, another alteration - p.Met 263Thr - had been previously reported in a homozygous fashion, giving rise to a severe variant form of MSUD[7]. In contrast, our patients with p.Met263Arg had a severe neonatal course of MSUD. The p.Ile401Thr variation occurred in one patient in a homozygous and in another patient in a heterozygous fashion. Both patients were reported to have classical MSUD with severe neonatal course.

Compilation of the Molecular Genetic Data of Communicated Turkish MSUD Patients

The mutational profile is heterogeneous in Turkish patients with MSUD. Two mutations - p.Cys258Tyr in the *BCKDHA* gene and p.Tyr383Stop in the *BCKDHB* gene - occurred three times, and five mutations - p.Thr151Met in the *BCKDHA* gene, p. Ala91Val and p.Arg285Stop in the *BCKDHB* gene, and p.Ile401Thr and p.Met263Arg in the *DBT* gene - occurred twice. The families were from 19 different cities/areas scattered throughout Turkey with the exception of the most southeastern part. There was no cluster for a single mutation except for p.Cys258Tyr in the *BCKDHA* gene, a hypothetical founder mutation in the Çamlıdere population[1].

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