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Mutational Spectrum of Maple Syrup Urine Disease in Spain

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Mutations in any of the three different genes BCKDHA, BCKDHB, and DBT encoding for the E1α, E1β and E2 catalytic components of the branched-chain α-ketoacid dehydrogenase (BCKD) complex can cause maple syrup urine disease (MSUD). The disease presents heterogeneous clinical and molecular phenotypes. Severity of the disease ranges from the classical to the mildest variant types. Here, we describe the MSUD genotypes and related phenotypes in a cohort of 33 Spanish patients. Based on complementation testing, we selected 15 patients as defective in E1β, 10 in E1α, seven in E2l; one remains unclassified. 92.5% of alleles have been characterized, and the mutational spectrum includes 36 different sequence variations presumably leading to loss-of-function, 15 changes in the BCKDHA, 14 in the BCKDHB, and seven in the DBT genes. Twenty-four changes are novel. The mutational profile is heterogeneous with no prevalent sequence variations detected, except for the E1β mutation, c.487G>T (p.Glu163X), which appears on six out of 30 disease alleles analyzed. Approximately 30% of the patients included in this study showed a variant MSUD phenotype. That included 50% of the patients identified as EIa and at least four out of seven of those selected as EII. Precise genotypes as c.[647C>T]+[889C>T] for the EIa and c.[827T>G]+[1349C>A] for the EII appeared associated to the mildest presentations of the disease. © 2006 Wiley-Liss, Inc.

KEY WORDS: MSUD; branched-chain α-ketoacid dehydrogenase complex; BCKDHA; BCKDHB; DBT

INTRODUCTION

Maple syrup urine disease (MSUD) is an autosomal recessively inherited disorder of branched-chain amino acid (BCAA) metabolism caused by the defective activity of branched-chain α-ketoacid dehydrogenase complex (BCKD). The blockage results in neurotoxic accumulation of leucine, valine, isoleucine and their respective α-keto acids in cells and body fluids (Chuang, 2001).

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The mammalian BCKD is a member of the highly conserved mitochondrial α-ketoacid dehydrogenase complexes. This catalytic machine is organized around a cubic core comprising 24 lipoate-bearing dihydrolipoyl transacylase (E2) subunit to which multiple copies of branched-chain α-ketoacid decarboxylase (E1), dihydrolipoamide dehydrogenase (E3), a specific kinase and a specific phosphatase responsible for the regulation of the BCKD complex are attached through ionic interactions (Pettit, et al., 1978; Reed, et al., 1985). The E1 component, whose crystal structure in humans has been determined to a 2.7 Å resolution (Ævarsson, et al., 2000), and refined to 1.8 Å (Wynn, et al., 2003), is a thiamine diphosphate dependent enzyme consisting of two E1α and two E1β subunits with two cofactor binding pockets located at the interface between α and β subunits. There are six genetic loci encoding subunits of the complex. The genomic changes that impair BCKD activity can occur in any of the catalytic components of complex, but both alleles at a single gene locus must harbor nucleotide changes. Genetic subtypes, based on the affected loci, are known as: type Ia (MIM# 608348) for *BCKDHA* gene (E1α subunit); type Ib (MIM# 248611) for *BCKDHB* gene (E1β subunit), type II (MIM# 248610) for *DBT* gene (E2 subunit) and type III (MIM# 238331) for mutations affecting the *DLD* gene (E3 subunit) (Chuang, 2001). The E3 component is used by other mitochondrial complexes included the three α-ketoacid dehydrogenase complexes, and the glycine cleavage system. Thus, mutations in this gene alter the function of the four different enzymes.

MSUD patients can be divided into different clinical phenotypes, ranging from the classical form, which account for 75% of MSUD patients, to mild variant types. Parameters such clinical presentation, residual enzyme activity in cells, leucine tolerance, and thiamine responsiveness have been used to classify the severity of the disease (Mitsubuchi et al., 2005). Except for type II and III MSUD (Chuang, et al., 1997; Chuang, et al., 2004; Fisher, et al., 1989), which are linked to the thiamine-responsive and E3-deficient phenotypes, respectively, a tight correlation between a specific genetic subtype and a particular phenotype of MSUD has not been demonstrated.

In the present study, which is the first large mutation report for Spanish MSUD patients collected through the national network, we examined the molecular phenotype and mutational spectrum of 33 patients and characterized 92.5% of the disease alleles. Twenty-four novel mutations have been identified and precise genotypes have been related to the mildest presentation of the disease.

MATERIAL AND METHODS

Subjects

This study includes 33 fibroblast cell samples from patients referred to our laboratory with clinical and/or biochemical diagnosis of MSUD. The cohort includes 30 Spanish cases, 2 gypsies from Spain and Portugal, and 1 child from Mauritania. Patients were categorized into classical and variant phenotypes based on BCKD deficiency assessed by measuring the L-[1-^{14}C] leucine decarboxylation rate by intact cells in absence of BCKD kinase inhibitors (Duran, et al., 1978), plasma amino acid levels, and mode of clinical presentation. The phenotypic evaluation was based on written documents sent by the referring clinicians. Informed consent was obtained from the referred institutions. Genetic subtypes Ia, Ib and II were identified by performing somatic complementation analysis. Patient cell strains were fused with fibroblast lines GM 00649A and GM 1364, representative of Ia and II MSUD molecular phenotypes (Coriell Cell Repositories, Candem, New Jersey, USA) (http://locus.umdnj.edu/nigms) in the presence of 50% PEG 4000, during 65 seconds. Complementation was monitored by measurement of $^{14}CO_2$ production after incubation of fibroblast in presence of L-[1-^{14}C] leucine. E3 deficiency was discarded after measuring the enzyme activity in fibroblast extracts (Chuang, et al., 1981).

Mutation analysis

Genomic DNA and total cellular RNA were extracted from fibroblast cultures. Mutation analysis was performed by direct sequencing of PCR fragments obtained after amplification of the entire coding sequence of *BCKDHA*, *BCKDHB* or *DBT* genes. The cDNAs fragments were obtained according to standard protocols using primers designed based on GeneBank cDNA sequences (accession numbers NM_000709.2 for *BCKDHA*, NM_000056.2 for *BCKDHB* and NM_001918 for *DBT*). To confirm the detected mutations we sequenced both strands (forward and reverse) of corresponding exons and their intronic flanking sequences. Primers to amplify the genomic DNA samples were designed according to GeneBank sequences. Direct cycle sequencing of all PCR fragments was performed with BigDye Terminator v 3.1 mix (Applied Biosystem, Foster City, CA. USA) (www.appliedbiosystems.com) and analyzed by capillary electrophoresis on an ABI Prism 3700 Genetic Analyzer (Applied Biosystem). All primers sequences are available on request.

The mutation nomenclature used follows the recommendation of the Human Genome Variation Society (http://www.hgvs.org/mutnomen). Genomic contigs NT_011109 for *BCKDHA* gene, NT_007299 for *BCKDHB* and NT_028050 for *DBT* were used for the genomic sequence. cDNA numbering commences from the ATG start codon, where +1 is the A of the ATG translation initiation codon.

Sequences of homologous proteins to human E1 and E2 components were obtained using BLAST (Altschul et al., 1990). Multiple alignments to analyze conservation were performed using ClustalW (Thompson et al., 1994).

Location of residues as well as generation of protein plot was performed using PyMOL (DeLano, 2002) deLano Scientific, San Carlos, CA9, and the three-dimensional coordinates of the Protein Data Bank, entry 1XY7.

RESULTS AND DISCUSSION

We have analyzed the *BCKDHA*, *BCKDHB*, and *DBT* genes from 33 MSUD patients mostly from Spain. Somatic cell complementation studies applied to the cell strains allowed their classification into the genetic subtypes Ia, Ib and II according to the gene presumable harboring the mutant alleles. The distribution observed, 15 /33 in the Ib subtype, 10 /33 in the Ia, 7/ 33 in the II and 1 with an ambiguous result is similar to that found in other studied populations (Henneke, et al., 2003; Nellis and Danner, 2001). No EIII genetic subtype strain has been detected in this cohort (data not shown).

Mutation detection

We have identified thirty-six nucleotide sequence variations that presumably lead to loss-of-function, including 15 changes in the *BCKDHA*, 14 in the *BCKDHB* and 7 in the *DBT* genes. Of them, twenty-four are novel and are distributed abroad the three genes as shown in Tables 1, 2 and 3. The rest corresponds to allelic variants previously described, mainly, in Hispanic patients (Chuang, et al., 1995; Henneke, et al., 2003). We have also detected three single polymorphic variants located in the encoded sequence of the *BCKDHA* gene. In all cases where parental DNA was available, inheritance was confirmed. Molecular data for all changes, including nucleotide variation, affected exon, protein prediction, occurrence at conserved amino acid are included in Tables 1, 2 and 3. In absence of expression studies, the pathogenicity of the novel missense mutations was assessed by discarding their presence in at least 100 Spanish control alleles. On the other hand, most of the variations described here, affect highly conserved residues between the human E1 or E2 component and their homologous proteins compared in 50 sequences available in the nucleotide databases from animal (including mammals, chicken, insects and parasites as plasmodium) and plant genomes, strengthening their impact on the structure/function of the proteins. In the remaining, the disease-causing effect was assumed when the alteration led to a premature termination codon (PTC).

In the *BCKDHA* gene, with no prevalent mutation identified, most of the allelic variants detected are clustered in exon 6 and exon 7 (Table 1) and could be predicted as missense changes except c.940C>T, causing p.Arg314X. According to the crystal structure of the human E1 component, residues included in this group are mainly located at subunit-subunit surfaces (Figure 1A) and pathogenicity could be the result of their detrimental effect on subunit-subunit interactions. So, the previously described p.Ala285Pro (Wynn, et al., 1998), p.Gly290Arg (Chuang, et al., 1995; Wynn, et al., 1998), and the novels p.Arg297Cys, p.Gly283Asp, p.Gly300Ser, p.Asp302Ala and p.Glu327Lys affect the α-α´ subunit interaction (Ævarsson, et al., 2000). Residues Ala253, Ala216, and Ala220 are all located in the same alpha helix close to the EIβ interface (Fig. 1A). The allelic variant c.1037G>A (p.Arg346Hys) affects a residue involved in the hydrogen-bonding network required to maintain the phosphorylation loop region necessary for the recognition by lip-LBD and channelling of substrate in the BCKD machine (Li, et al., 2004). Moreover, we have detected three frameshift mutations, c.117delC, already described in other population (Chuang, et al., 1994), and the novel, c.117dupC and c.1233delC1242_1243ins27. The last one deletes a C at position 1233 and inserts the sequence GTGTATCAG, in triplicate, disrupting the open reading frame. This results in the elimination of the stop codon at position 446 and the subsequent introduction of a downstream stop codon. The resulting protein would predictably contain 37 additional amino acids. Finally, we have also identified a patient carrying a sequence variation not yet characterized at the genomic level but causing the skipping of exon 6 (r.647_853del) when it was studied at cDNA level. In addition to these pathogenic substitutions, we have found the silent variants already reported, c.116C>A (p.Pro39His) (Henneke, et al., 2003), c.972T>C (p.Phe324Phe) (Dursun, et al., 2002), and c.1221G>A (p.Leu407Leu) (HGVbase SNP000008700). These apparently neutral variations may be helpful in allele tracking when skipping or deletion is suspected.

In the *BCKDHB* gene, distribution of nucleotide changes includes nine nucleotide substitutions, with predicted effects on protein of nonsense or missense mutations, four deletions, and one duplication (Table 2). According to the

crystal structure of the E1 component, residues Gly135, Val130, Arg168, and Gly172 are all located at the α-β'subunit interface (Figure 1B). Deletion of Val 130, located in a cluster of hydrophobic residues (Ile147, Ile160 and Ala156), could modify the structure of its containing alpha helix which is in close contact with the EIα monomer. Residues Arg216, Ile214 and Val251 are all located at the inner core of the β-subunit, and the corresponding changes could compromise the stability of the region. Finally, a slipped-strand misspairing mechanism could be responsible for the presence of two different mutations c.92_102del11 and c.92_102dup11 in a recurrent sequence region of the gene. In this gene, three sequence variations, c.487G>T (p.Glu163X), c.595_596delAG (p.Pro200X) and c.799C>T (p.Gln267X) appear to be particularly prevalent with frequencies of 6/30, 4/30 and 3/30 respectively (Table 2). The c.487G>T change appears in homozygous fashion in three patients without consanguineous pedigree proved but all coming from the same Spanish geographic area.

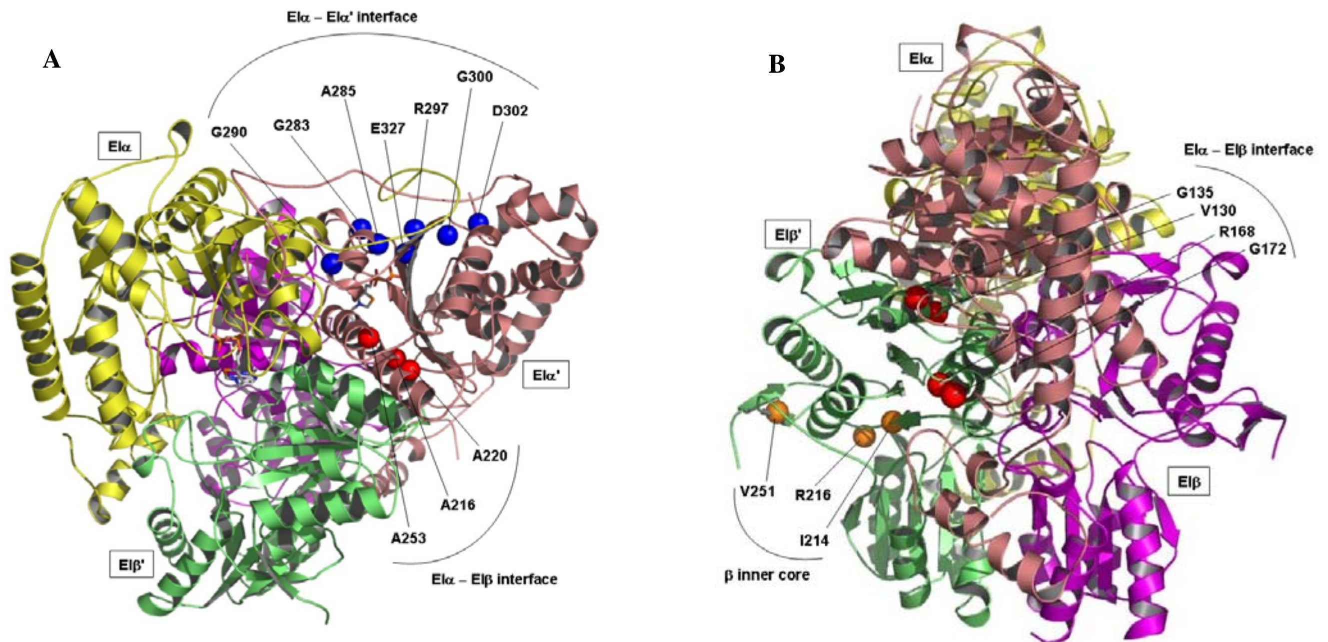

Figure 1. A: Ribbon-plot representation of four subunits in EI (Ævarsson et al., 2000), numbered as EIα, EIα', EIβ, and EIβ', respectively, including location of some mutated residues in EIα chain. Residues are clustered according to their position in EIα-EIα'interface (blue spheres) or EIα-EIβ interface (red spheres). **B:** Positioning of mutated residues in EIβ chain, clustered in the EIα-EIβ interface (red spheres) or in the EIβ inner core (orange spheres). Plot was generated using PyMOL (W.L. DeLano (2002) DeLano Scientific, San Carlos, CA).

Table 1. Variations detected in the *BCKDHA* gene

Exon	Nucleotide change	Protein prediction	Conservation	Protein domain	Frequency	Reference
2	c.117delC	p.Arg40fs62X			1/20	This study
2	c.117dupC	p.Arg40fs50X			1/20	(Chuang, et al., 1994)
6	c.647C>T	p.Ala216Val	Identical orthologs	α-β´interface	1/20	This study
	r.647_853del[a] (exon 6)	p.Val212_Ala247delAla248fsX6				This study
6	c.659C>T	p.Ala220Val	Identical orthologs	α-β´interface	2/20	This study
6	c.757G>A	p.Ala253Thr	Identical orthologs	α-β´interface	1/20	(Nobukuni, et al., 1993)
6	c.848G>A	p.Gly283Asp[b]	Identical orthologs	α-α´interface	1/20	This study
6	c.853G>C	p.Ala285Pro	Identical orthologs	α-α´interface	2/20	(Wynn, et al., 1998)
7	c.868G>A	p.Gly290Arg	Identical orthologs	α-α´interface	2/20	(Chuang, et al., 1995)
7	c.889C>T	p.Arg297Cys	Identical orthologs	α-α´interface	1/20	This study
7	c.898G>A	p.Gly300Ser[b]	Identical orthologs	α-α´interface	1/20	This study
7	c.905A>C	p.Asp302Ala	Identical orthologs	α-α´interface	2/20	This study
7	c.940C>T	p.Arg314X			1/20	This study
7	c.979G>A	p.Glu327Lys		α-α´interface	1/20	This study
8	c.1037G>A	p.Arg346His	Identical orthologs		1/20	This study
9	c.1233delC1242_1243ins27	p.Asp411fs			2/20	This study

DNA mutation numbering is based on cDNA reference sequence (GenBank Accession number NM_000709.2) considering nucleotide +1 as the A of the ATG translation initiation codon.

[a]The nucleotide sequence variations have not yet been identified, at the genomic level. At cDNA level, the effect is the skipping of exon 6.

[b]Both mutations have been identified on the same mutant chromosome.

Table 2. Variations detected in the *BCKDHB* gene

Exon	Nucleotide change	Protein prediction	Conservation	Protein domain	Frequency	Reference
1	c.92_102del11	p.Leu31fs			1/30	(Nobukuni, et al., 1991; Parrella, et al., 1994)
1	c.92_102dup11	p.Phe35fs			1/30	This study
4	c.348delA	p.Lys116fs			1/30	This study
4	c.389_391delTTG	p.Val130del		α-β interface	2/30	This study
4	c.403G>A	p.Gly135Arg	Identical orthologs and paralogs (ODPB)	α-β interface	1/30	(Henneke, et al., 2003)
5	c.487G>T	p.Glu163X			6/30	This study
5	c.503G>A	p.Arg168His	Identical orthologs	α-β interface	2/30	(Henneke, et al., 2003)
5	c.514G>T	p.Gly172Trp	Identical orthologs Semiconserved in paralogs	α-β interface	1/30	This study
5	c.595_596delAG	p.Pro200X			4/30	(Henneke, et al., 2003)
6	c.641T>A	p.Ile214Lys	Semiconserved in paralogs (Ile/Val/Met/Ala)	β-inner core	2/30	This study
6	c.646A>G	p.Arg216Gly	Semiconserved in orthologs (Arg/Ser)	β-inner core	2/30	This study
7	c.752T>C	p.Val251Ala	Identical orthologs	β-inner core	1/30	(Nellis and Danner, 2001)
7	c.799C>T	p.Gln267X			3/30	(Nellis, et al., 2003)
8	c.853C>T	p.Arg285X			2/30	(Henneke, et al., 2003)

DNA mutation numbering is based on cDNA reference sequence (GenBank Accession number NM_000056.2) considering nucleotide +1 as the A of the ATG translation initiation codon.

Table 3. Variations detected in *DBT* gene

Exon	Nucleotide change	Protein prediction	Conservation	Protein domain	Frequency	Reference
2	c.75_76delAT	p.Cys26fsX1			1/14	(Fisher, et al., 1993)
4	c.394G>A	p.Gly132Arg	Identical orthologs	Lipoyl-binding site	1/14	This study
6	c.754_760del	p.Lys252_Thr253delGlu254fs			1/14	This study
7	c.788T>C	p.Met263Thr	Identical orthologs	Inner core	2/14	This study
7	c.827T>G	p.Phe276Cys	Identical orthologs	Inner core	3/14	(Fisher, et al., 1991)
11	c.1349C>A	p.Ala450Asp	Identical orthologs	Inner core	1/14	This study
11	c.1385G>C	p.Arg462Pro	Identical orthologs	Inner core	4/14	This study

DNA mutation numbering is based on cDNA reference sequence (GenBank Accession number NM_001918) considering nucleotide +1 as the A of the ATG translation initiation codon.

Seven different variations have been found in the *DBT* gene. Two of those correspond to deletions, and the rest are nucleotide substitutions, with predicted effect of missense mutations, mostly affecting residues located at the E2 inner core domain (Table 3). Crystal structure has not yet been determined. In this gene, changes, c.827T>G and c.1385G>C, account for 50% of the studied alleles. The c.1385G>C variation appears in one homozygous patient with known consanguinity and in heterozygous fashion in other two patients, all from the south eastern of Spain.

Intronic polymorphism c.996-33delc in the *BCKDHA* gene, and c.344-24c>t in the *BCKDHB* gene have been also identified. Patients carrying these polymorphisms are included in Table 4.

Phenotype and mutation profile

We have categorized our cohort of MSUD patients into classic and variant forms based on indirect biochemical and clinical parameters such as BCKD activity, plasma L-alloisoleucine/isoleucine (aleu/ileu) ratio under metabolic control as a measure of BCAA tolerance (Wendel, et al., 1989), onset of symptoms and outcome. However, differences between phenotypes are not sufficiently defined. Thus, variants exist also as a gradation from a more severe to a mildest presentation of the disease. On the other hand, we have found inconsistencies between biochemical parameters as BCKD deficiency and clinical phenotype likewise as previously described (Nobukuni, et al., 1991). That is the case of the classical Ib patients, 7720, 5387, and 15919 (Table 4), all harboring the same mutation in homozygous fashion but with residual BCKD activity, ranging from <1 to 13%. On the other hand, several patients were detected in newborn screening programs (Table 4). This has the effect of minimizing consequences of the blockage but also masks features that may be related to the genotype.

As it is shown in Table 4, 50% of the Spanish patients having molecular phenotype Ia correspond to the variant form of the disease. Precise novel mutations detected in *BCKDHA* as c.647C>T (p.Ala216Val), c.659C>T (p.Ala220Val), and c.889C>T (p.Arg297Cys), seem to be responsible for the selection as variants of those patients. This is the case of patient 18804 (c.[647C>T]+[889C>T]), who displayed the highest residual BCKD activity detected and a very mild clinical course of the disease. Patients 16392 and 8092, that showed a more severe course of the disease, were both compound heterozygous carriers for the nucleotide variation c.659C>T associated with the 853G>C and 905A>C changes respectively, which were both detected in the classic MSUD patient 11486. It is also interesting to show that the sequence change c.868G>A (p.Gly290Arg), previously described as Gly245Arg in three intermediate MSUD patients from Mexico (Chuang, et al., 1995), appears in our cohort only on two alleles of two more severe variant MSUD. The non-prevalence of this mutation in Spain precludes its possible ethnic origin as was previously suggested (Chuang, et al., 1995).

In line with previous reports (Chuang, et al., 1997; Chuang, et al., 2004; Tsuruta, et al., 1998), most of the EII MSUD Spanish patients show a milder phenotype of the disease (Table 4) and have been considered as variants. It is interesting to remark that the mutation pattern identified in this group correspond to missense mutations, affecting residues in a conserved core of the E2 protein. According to clinical and biochemical data, only two EII patients could be unequivocally selected as thiamine-responsive, both carrying the c.827T>G (p.Phe276Cys) change in heterozygous fashion. This mutation has been previously described as F215C and identified in patients with a thiamine-responsive phenotype (Chuang, et al., 1997); (Chuang, et al., 2004). For the remaining patients, responsiveness has not been established. The only EII patient with a clear classical phenotype of the disease, 16890, carried the frameshift mutation c.754_760del combined with the novel missense c.394G>A affecting a residue located in the putative lipoyl-binding site of the protein. This underlies the importance of this region for the total catalytic activity of the complex.

Patients included in the Ib genetic subtype, all carrying mutations in the *BCKDHB* gene have a classical phenotype. In concordance with the phenotype severity, a large amount of changes with predicted effect of PTC have been identified in these series.

Finally, in patient 10848, who shows a mild phenotypic expression of the disease, the three cDNAs, *BCKDHA*, *BCKDHB* and *DBT* have been analyzed but we have not detected any variations in any of the three genes susceptible to be pathogenic. Further studies, including prospecting of other genetic subtypes are currently underway. Moreover, the very recently reported expression control of BCKD in cells by human microRNA (miR29b) (Mersey et al, 2005) offers a new approach to investigate this type of variant form.

Table 4. Clinical, biochemical, and molecular data of Spanish MSUD patients

Patient	Genetic subtype	Genotype	BCKD activity (% of normal)	Aleu/ileu	Onset	Clinical phenotype	Outcome/age
16243[f]	Ia	c.[117delC]+[117dupC]	<1	2.17	Neonatal	classic	LT 1,5y; Slight PMR/5y
20493[a f]	Ia	c.[757G>A]+[848G>A;898G>A]	<1	0.98	Neonatal	classic	NPD/1y
11486[a]	Ia	c.[853G>C]+ [905A>C]	<1		Neonatal Screening	classic	LT at ?/13y
5888	Ia	c.[940C>T]+[1037G>A]	4.4		Neonatal	classic	Died 10m
141966[i]	Ia	c.[1233delC1242_1243ins27]+ [1233delC1242_1243ins27]	<1		Neonatal	classic	Died 18m
18804[f]	Ia	c.[647C>T]+[889C>T]	30	0.1	Neonatal Screening	variant	Asymptomatic, no protein restrict./3y
16392[af]	Ia	c.[659C>T]+ [853G>C]	4		Neonatal Screening (TMS)	variant	?/4y
8092[b]	Ia	c.[659C>T]+ [905A>C]	5.7	0.52	Infantil 6m	variant	No metab decomp/ Died 11y after surgery
7469	Ia	c.[868G>A]+[979G>A]	2.5	0.77	Infantil 3m	variant	PMR/23y
16115[a d]	Ia	[c.868G>A]+ [r.647_853del]	3.2	0.89	Neonatal	variant	NPD; LT 2y/5y
14651	Ib	c.[92_102del11]+[403G>A]	<1	3.04	Neonatal	classic	LT 1.5y/7y

12279[f]	Ib	c.[92_102dup11]+[799C>T]	11	1.1	Neonatal	classic	PMR/11y
17340[f]	Ib	c.[348delA]+[514G>T]	1.3		Neonatal	classic	?/3y
16259[f g]	Ib	c.[389_391delTTG]+[389_391delTTG]	1.7	0.89	Neonatal	classic	NPD; LT 1.5y/4y
5387	Ib	c.[487G>T]+[487G>T]	3.9		Neonatal	classic	Died 6m
7720[c]	Ib	c.[487G>T]+[487G>T]	<1		Neonatal	classic	Lost
15919[f]	Ib	c.[487G>T]+[487G>T]	13		Neonatal	classic	PMR/5y
13981[f]	Ib	c.[503G>A]+[503G>A]	<1	0.7	Neonatal	classic	PMR/16y
9026	Ib	c.[595_596delAG]+[595_596delAG]	1		Neonatal	classic	Lost
16042[e f]	Ib	c.[595_596delAG]+[799C>T]	<1	1.08	Neonatal	classic	LT 2y; slight PMR /5y
9012	Ib	c.[595_596delAG]+[799C>T]	<1	1.48	Neonatal	classic	Died 8y
20426[f]	Ib	c.[641T>A]+[641T>A]	1.2		Neonatal Screening (TMS)	classic	?/1y
16065[h]	Ib	c.[646A>G]+ [646A>G]	<1	0.7	Neonatal	classic	PMR/6y
11162[e]	Ib	c.[752T>C]+ [?]	<1	1.5	Neonatal	classic	LT1.5y/14y
16757[f j]	Ib	c.[853C>T]+ [853C>T]	2.5	1	Neonatal	classic	PMR/4y
8300	II	c.[75_76delAT]+[1385G>C]	2.8		Neonatal	classic?	Lost
16890[f]	II	c.[394G>A]+[754_760del]	1.3	0.92	Neonatal	classic	PMR/died 3y after LT

9942	II	c.[788T>C]+[788T>C]	6	<0.16	Infantil	variant	No metab decompen/17y
16800[f]	II	c.[827T>G]+[1385G>C]	7.7	0.18	Neonatal Screening	variant	Doing well/5y
13152	II	c.[827T>G] +[?]	6	No detectable	Infantil 15m	thiamine responsive	Asymptomatic/ 11y
15100	II	c.[827T>G]+[1349C>A]	3.5	0.10	Prenatal study	thiamine responsive	Asymptomatic/ 6y
21639[g]	II	c.[1385G>C]+[1385G>C]	19		Neonatal		Wilson disease; PMR/ 7y
10848[c d]			10	0.21	Neonatal Screening	variant	Asymptomatic/ 15y

BCKD activity measured in intact fibroblasts from MSUD patients and normal individuals with L-[1-^{14}C]leucine in absence of BCKD kinase inhibitors, aleu/ileu ratio: mean of values obtained under metabolic control, LT: liver transplantation, TMS: tandem mass screening, PMR: psychomotor retardation, NPD: normal psychomotor development.

[a] *BCKDHA* polymorphic variations c.972T>C (p.Phe324Phe) and c.1221G>A (p.Leu407Leu) carried in heterozygous fashion.

[b] *BCKDHA* polymorphic variations c.972T>C (p.Phe324Phe) and c.1221G>A (p.Leu407Leu) carried in homozygous fashion.

[c] *BCKDHA* polymorphic variation c.116C>A (p.Pro39His) detected in heterozygous fashion.

[d] *BCKDHA* polymorphic variation 996-33delc carried in heterozygous fashion.

[e] *BCKDHB* polymorphic variation 344-24c>t carried in heterozygous fashion.

[f] Mendelian inheritance confirmed in parents.

[g] Known consanguinity.

[h] From Mauritania.

[i] Gypsy from Spain.

[j] Gypsy from Portugal.

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