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Fourteen new mutations of *BCKDHA*, *BCKDHB* and *DBT* genes associated with maple syrup urine disease (MSUD) in Malaysian population

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ABSTRACT

Maple syrup urine disease (MSUD) is a rare autosomal recessive metabolic disorder. This disorder is usually caused by mutations in any one of the genes; *BCKDHA*, *BCKDHB* and *DBT*, which represent E1α, E1β and E2 subunits of the branched-chain α-keto acid dehydrogenase (BCKDH) complex, respectively. This study presents the molecular characterization of 31 MSUD patients. Twenty one mutations including 14 new mutations were identified. The *BCKDHB* gene was the most commonly affected (45.2%) compared to *BCKDHA* gene (16.1%) and *DBT* gene (38.7%). *In silico* webservers predicted all mutations were disease-causing. In addition, structural evaluation disclosed that all new missenses in *BCKDHA*, *BCKDHB* and *DBT* genes affected stability and formation of E1 and E2 subunits. Majority of the patients had neonatal onset MSUD (26 of 31). Meanwhile, the new mutation; c.1196C > G (p.S399C) in *DBT* gene was noted to be recurrent and found in 9 patients. *Conclusion*: Our findings have expanded the mutational spectrum of the MSUD and revealed the genetic heterogeneity among Malaysian MSUD patients. We also discovered the p.S399C from *DBT* gene was noted as a recurrent mutation in Malay community and it suggested the existence of common and unique mutation in Malay population.

1. Introduction

Maple syrup urine disease (MSUD, OMIM #248600) is an autosomal recessive inherited metabolic disorder, caused by the impaired function of branched-chain α-ketoacidehydrogenase (BCKDH) complex [1]. MSUD clinical phenotype can be divided into 5 types based on clinical presentation and severity; classical, intermediate, intermittent, thiamine-responsive and E3-deficient forms [1].

The BCKDH complex consists of three catalytic components which are organized around a cubic core of 24 identical dihydrolipoamide branched-chain transacylase (E2) that binds to multiple subunits of BCKA dehydrogenase/decarboxylase (E1) and dihydrolipoamide dehydrogenase (E3) [2,3]. BCKD kinase and mitochondrial-targeted protein phosphatase (PP2Cm) are regulatory enzymes subunits that also attached to the complex. The E1 catalytic component forms as a heterotetramer of E1α and E1β subunits in BCKDH complex [2,3]. Molecular MSUD genotype has been described in catalytic subunit of BCKDH complex; where for type 1A (OMIM# 608348), mutations occurs in *BCKDHA* gene (E1α subunit); for type 1B (OMIM# 248611) specifically for mutations found in *BCKDHB* gene (E1β subunit), meanwhile for type II (OMIM# 248610), mutations affecting the *DBT* gene (E2 subunit) and type III (OMIM # 238331) for mutations occurs in *DLD* gene (E3 subunit) [1].

MSUD is known as pan-ethnic disorder and world-wide incidence is very rare, estimated 1:185000. However, in the Mennonites population in Pennsylvania, the incidence is reported to be very high, at 1 in 176 births [1]. In Malaysia, inborn error metabolism screening programme successfully detected 25 patients were MSUD and most of the patients occurred in Malay ethnic [4].

Molecular genetic testing is essential to facilitate diagnosis and treatment. In addition, information from this testing is important for genetic counseling of the affected family. Genomic alterations that affect BCKDH activity can occur in any of the E1, E2 or E3 subunits, however both alleles at a single gene locus must have mutations [1,5]. To date, > 283 disease-causing mutations have been described in Human Gene Mutation Database (HGMD) (http://www.hgmd.cf.ac.uk/ac/index.php) and scattered over the *BCKDHA*, *BCKDHB* or *DBT* genes. Mutations in *BCKDHA* and *BCKDHB* gene are commonly reported in patients with MSUD compared to *DBT* gene. MSUD patients can be affected either as homozygous or compound heterozygous genotypes. Here, we present the molecular characterization of 31 patients with MSUD by analyzing the *BCKDHA*, *BCKDHB* and *DBT* genes.

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2. Materials and methods

2.1. Patients

31 patients from 25 unrelated families who attended Metabolic Clinic, Kuala Lumpur Hospital (HKL) were studied. All of them were diagnosed to have MSUD based on clinical presentations and biochemical testing by using quantitative plasma amino acid analysis [4]. All patients have been referred to Molecular Diagnostics and Protein Unit (UMDP) for molecular genetic testing. If mutation was detected in the patient, parent samples were requested to analyze the presence of similar mutation in order to test the inheritance cases. Samples from parents were available only for 18 patients and all parents were healthy. 50 healthy unrelated individuals born in Malaysia were also included as control group in this study. Informed consent was acquired from the participants before approximately 5 to 10 ml of peripheral blood was collected in EDTA tubes. This study was approved by the Medical Research and Ethics Committee (MREC) (approval ID: NMRR-12-1321-13062) and it was performed according to the Declaration of Helsinki.

2.2. PCR and sequencing

Genomic DNA of patients and appropriate family members, and also healthy normal control was isolated from a peripheral blood leukocyte using the QIAamp DNA Blood Mini Qiacube Kit (QIAGEN, Hilden, Germany). The touchdown polymerase chain reaction (PCR) method was used to amplify all three genes, *BCKDHA*, *BCKDHB* and *DBT* including intron-exon boundaries [6]. All primers were tagged with the universal M13 sequence and detailed description was available upon request. The presence of amplicons with the expected size was run on 1.5% agarose gel under standard electrophoresis parameters.

The QIAquick PCR purification kit (QIAGEN, Hilden, Germany) was used to purify the PCR product and the purified product was subsequently preceded to the cycle sequencing. Big Dye Terminator cycle sequencing version 3.1 (Applied Biosystems, Foster City, CA), was then used to run the bi-directional sequencing while purify cycle sequencing product was carried out using DyeEx 2.0 Spin kit (QIAGEN, Hilden, Germany). Finally, the sequencing reaction was performed on a 48-channel capillary ABI 3730 Genetic Analyzer.

2.3. PCR for large deletion

PCR to detect the large deletion mutations in *BCKDHB* and *DBT* genes were performed by using specific primers. To detect the deletion of Exon 1 in *BCKDHB* gene, specific primers: BCKDHB-1F (forward) 5' GTAAAACGACGGCCAGTGCATTTTAAGGAGGGTGTGAA 3′ and BCKDHB-1R (reverse) 5' GCGGATAACAATTTCACACAGGAATAAGCTG GGATGCAAGGA 3′ were used. While to detect the deletion of Exon 11 in *DBT* gene, specific duplex PCR primers described by Chi and colleagues [7] were used. The product was analyzed on 2% agarose gel and visualized by ethidium bromide staining.

2.4. Mutation confirmation

The sequencing results were compared with the genomic reference sequence and coding DNA reference sequence of *BCKDHA* (NC_000019.9 and NM_000709.3), *BCKDHB* (NC_000006.11 and NM_183050.2) and *DBT* (NC_000001.10 and NM_001918.3) using SeqScape Software version 2.5 (Applied Biosystems, Foster City, CA). All reference sequences were retrieved from human genome GRCh37/hg19. Mutation surveyor Software (Softgenetics, State College, PA) was then used to reconfirm the small insertion or deletion mutation. Human Genome Variation Society (HGVS) (http://www.hgvs.org/mutnomen) was referred to report the single nucleotide variants (SNVs), insertion and deletion.

The publicly available databases: HGMD [8] and 1000G project (http://www.1000genomes.org) [9] were used for comparison with identified mutations. Meanwhile, in order to rule out a polymorphism, all new mutations were also analyzed with 50 healthy normal individuals (100 alleles). In addition, all new mutations were also compared with variants included in the Genome Aggregation Database (gnomAD) [10].

2.5. In silico webservers and structure prediction

Multiple sequence alignments were performed using HomoloGene database (http://www.ncbi.nlm.nih.gov/homologene) to verify the degree of conservation. The pathogenicity of the DNA alterations was then evaluated using three *in silico* webservers, PolyPhen2 [11], SIFT [12] and Mutation Taster2 [13]. Mutation Taster2 was also used to predict clinical significant of the splice site, deletion and insertion mutation. The stability/instability of the protein was predicted based on I-Mutant 2.0 server [14].

Computational modeling was carried out to observe the effect of new missense mutations on protein structure. Crystal structures for *BCKDHA*, *BCKDHB* and *DBT* proteins were obtained from the protein data bank (RCSB-PDB) [15]. Human X-ray crystal structure was only available for *BCKDHA* and *BCKDHB* proteins. Human X-ray (PDB ID: 1X7Y) crystal structure with resolution of 1.57 Å was used as a template [16]. At the moment, the complete three-dimensional (3D) model of human *DBT* protein was not available in the protein database. Thus, X-ray bovine crystal structure (PDB ID: 2II3) [17] with resolution of 2.17 Å was selected as a template. At the moment, only 234 amino acids out of 482 amino acids (from amino acid 188 to 421) of bovine E2 subunit have been crystallized. The 3D structure of target human protein was built using MODELLER 9.9 [18,19] based on the bovine structure. Generation of mutation plots and visualization of protein effect were performed using PyMOL software [20].

3. Results

Thirty-one patients from 25 families were enrolled in this study. Among them, 28 patients (90.3%) were Malays and remaining three (9.7%) were Chinese. Twenty-six (83.9%) patients were clinically diagnosed with the neonatal onset classical form of MSUD, three (9.7%) were intermediate MSUD and two (6.4%) patients were intermittent phenotype. Information on clinical features of all 31 patients is presented in Table 1.

3.1. Molecular analysis in BCKDHA, BCKDHB and DBT genes

Molecular analysis successfully revealed that all patients carried mutations on both alleles, with 20 (64.5%) patients were homozygous and 11 (35.5%) patients were compound-heterozygous. Table 1 show the *BCKDHB* was found to be the most commonly mutated gene (14 patients, 45%), followed by *DBT* gene (12 patients, 38.7%) and *BCKDHA* gene (5 patients, 16%). The most common mutational types were missense (57%) followed by nonsense mutations (19%), splice site (9.5%), frame-shift (5%) and large deletion (9.5%).

Table 2 shows from the total of 21 mutations, 14 were identified as new mutations and not reported previously in HGMD and 1000G project. Meanwhile, 7 mutations have previously been reported in the literature. In order to delineate the pathogenicity of these 14 new mutations, each of them was checked with 50 healthy normal individuals. None of these 14 new mutations were detected in normal population. In addition, 12 new mutations were also not detected in gnomAD database. However not for the other 2 new mutations (T273I and R387*), which were found as heterozygous state in this database with alleles frequencies were 0.00001628% (4/245752 chromosomes) and 0.00003657% (9/246076 chromosomes), respectively. Missense mutations in *BCKDHA* and *BCKDHB* genes were mapped on the E1α and E1β

Table 1
Genotype-phenotype correlation of the 31 patients.

Family/ *Patient	Sex	Age of onset	Ethnic	Clinical Phenotype	Mutation state	Genetic subtype	Exon/ Intron	Mutation			Type of Mutation
								Genome number (GRCh37/ hg19)	Coding DNA number	Protein number	
A-1	F	2y	Malay	ITD	HM	E1α	Ex-8	g. 41928994C > T	c.1087C > T	p.R363W	Missense
B-1[a]	F	1y	Malay	ITD	HM	E1α	Ex-5	g.41925082C > T	c.527C > T	p.A176V	Missense
B-2	M	1y		ITD	HM						
C-1	F	8d	Malay	C	HM	E1α	Ex-4	g.41920009G > A	c.431G > T	p.S144I	Missense
D-1	M	6d	Malay	C	HM	E1α	Int1	g.41903846 T > C	c.108 + 6 T > C	p.?	Splice site
E-1	F	8d	Chinese	C	CHT	E1β	Ex-7	g.80910726C > T	c.818C > T	p.T273I	Missense
							Ex-10	g.81053501C > T	c.1159C > T	p.R387*	Missense
F-1	M	7 m	Chinese	ITM	CHT	E1β	Ex-3	g.80838904G > A	c.301G > A	p.G101S	Missense
F-2	F	NS		ITM	CHT		Ex-5	g.80878695T > G	c.581T > G	p.L194R	Missense
G-1	F	5d	Malay	C	HM	E1β	Ex-9	g.80982916C > T	c.1016C > T	p.S339L	Missense
H-1	F	6d	Malay	C	CHT	E1β	Ex-1	g.80816606G > T	c.196G > T	p.G66W	Missense
							Ex-9	g.80982916C > T	c.1016C > T	p.S339L	Missense
I-1[a]	F	7d	Malay	C	HM	E1β	Ex-3	g.80838934C > T	c.331C > T	p.R111*	Nonsense
I-2[b]	M	NS		C	HM						
I-3	M	NS		C	HM						
J-1	F	6d	Malay	C	CHT	E1β	Ex-10	g.81053436C > G	c.1094C > G	p.T365R	Missense
							Ex-10	g.81053501C > T	c.1159C > T	p.R387*	Nonsense
K-1	M	7d	Malay	C	CHT	E1β	Ex-2	g.80837330A > G	c.263A > G	p.D88G	Missense
							Ex-9	g.80982916C > T	c.1016C > T	p.S339L	Missense
L-1	F	5d	Malay	C	CHT	E1β	Ex-7	g.80910689T > C	c.781T > C	p.S261P	Missense
							Ex-7	g.80910710G > T	c.802G > T	p.E268*	Nonsense
M-1	F	5d	Malay	C	NA	E1β	5'UTR-Ex-1	g.(80819375_80816718)del	~ 3.3 kb deletion spanning 5'UTR to Exon 1	p.?	Large deletion
N-1	M	10d	Malay	C	NA	E1β	5'UTR-Ex-1	g.(80819375_80816718)del	~3.3 kb deletion spanning 5'UTR to Exon 1	p.?	Large deletion
O-1	M	5d	Malay	C	NA	E1β	5'UTR-Ex-1	g.(80819375_80816718)del	~ 3.3 kb deletion spanning 5'UTR to Exon 1	p.?	Large deletion
P-1	M	4d	Malay	C	HM	E2	Ex-9	g.100672014C > G	c.1196C > G	p.S399C	Missense
Q-1 [a]	F	5d	Malay	C	CHT	E2	Ex-9	g.100672143_100672144insT	c.1066_1067insT	p.V356Cfs*3	Frameshift
Q-2	F	NS		C	CHT		Ex-11	g.100661878C > G	c.1382C > G	p.S461*	Nonsense
R-1	F	4d	Malay	C	HM	E2	Ex-9	g.100672014C > G	c.1196C > G	p.S399C	Missense
S-1	M	5d	Malay	C	HM	E2	Ex-9	g.100672014C > G	c.1196C > G	p.S399C	Missense
T-1	M	NS	Malay	C	CHT	E2	Ex-9	g.100672014C > G	c.1196C > G	p.S399C	Missense
							Int1	g.100715321C/T	c.51 + 5 G > C	NA	Splice site
U-1[c]	M	7d	Malay	C	HM	E2	Ex-9	g.100672014C > G	c.1196C > G	p.S399C	Missense
U-2[c]	M	7d		C	HM						
V-1	M	4d	Malay	C	HM	E2	Ex-9	g.100672014C > G	c.1196C > G	p.S399C	Missense
W-1	F	6d	Malay	C	HM	E2	Int10–3'UTR Ex-11	g.100663570_10066831del	4.7-kb deletion flanking parts of intron 10 and the 3'UTR of exon 11	p.?	Large deletion
X-1	M	8d	Malay	C	CHT	E2	Ex-9	g.100672143_100672144insT	c.1066_1067insT	p.V356Cfs*3	Frameshift
							Ex-9	g.100672014C > G	c.1196C > G	p.S399C	Missense
Y-1	M	6d	Malay	C	HM	E2	Ex-9	g.100672014C > G	c.1196C > G	p.S399C	Missense

Abbreviations: [a] = sister, [b] = brother, [c] = Twins, F = female, M = Male, y = years, d = day, m = month, NS = neonatal screening, ITD = Intermediate, C = Classical, ITM = Intermittent, HM = homozygous, CHT = compound heterozygous, NA = not applicable.
Samples from parents were available for B, C, E, F, G, H, I, J, P, Q, R, S, T, U, V, W and Y.

subunits (Fig. 1a), while missense mutation in *DBT* gene was mapped on human E2 subunit model (Fig. 1b).

3.2. *In silico prediction and structural analysis for new mutations*

3.2.1. *BCKDHA gene*

One missense mutation was identified, leading to the amino acid change p.A176V. According to multiple sequence alignment analysis, A176 was 75% conserved among the species. 2 (SiFT and Mutation taster) out of 3 *in silico* servers predicted substitution from alanine to valine was disease-causing (Table 2). Meanwhile, PolyPhen server predicted this mutation as benign. Structural analysis exhibited A176 was located on the α-helix 5 of the α-subunit, near to the β-subunit. The backbone nitrogen of A176 was observed to form hydrogen bond interaction with backbone carbonyl oxygen of E172. Meanwhile, the carbonyl oxygen of A176 formed hydrogen bond interaction to backbone nitrogen of G180 and N181. The substituted V176 however did not alter the hydrogen bond interactions with their neighboring residues (Fig. 2a). However, I-Mutant server predicted this mutation affect the stability of the structure. Another new mutation was located near to splice donor site (c.108 + 6 T > C) and Mutation Taster webserver predicted this mutation was disease-causing.

3.2.2. *BCKDHB gene*

Two new nonsense (p.E268* and p.R387*) and 6 new missense (p.G66 W, p.D88G, p.G101S, p.L194R, p.S261P, p.T273I and p.T365R) mutations were identified and scattered in exons 1, 2, 3, 5, 7, 9 and 10 (Table 2). All nonsense and missense mutations showed high evolutionary conservation across orthologous E1β subunits (77% to 100%). Further *in silico* analyses predicted that all missense mutations in this gene were damaging to the protein function (Table 2).

Mutation p.G66W is located on the N-terminal domain of β subunit.

Table 2
In-silico servers prediction, population frequency, clinical phenotype of patients and co-segregation of the alleles in family.

Patient	Genetic subtype	Exon/ Intron	Coding DNA number	Protein number	*In-silico* prediction					Screening with 100 normal alleles	Population allele frequency from gnomAD database (Allele Frequency-%)	Clinical phenotype	Co-segregation alleles in family	Reference
					PolyPhen-2.0	SiFT	Mutation taster	I-Mutant	Gene Conservation in Eukaryota (%)					
A-1	E1α	Ex-8	c.1087C > T	p.R363W	-	-	-	-	-	-	-	ITD	NA	[38]
B-1	E1α	Ex-5	c.527C > T	p.A176V	B	D	DC	Dec	75	A	A	ITD	1 HM sibling, HT mother and HT father	This study
C-1	E1α	Ex-4	c.431 G > T	p.S144I	-	-	-	-	-	-	-	C	HT mother and HT father	[23]
D-1	E1α	Int1	c.108 + 6 T > C	p.?	-	-	-	-	-	A	A	C	NA	This study
E-1	E1β	Ex-7	c.818C > T	p.T273I	PD	D	DC	Inc	100	A	P *(1.628×10^{-5})	C	HT father	Both mutations detected in gnomAD database
		Ex-10	c.1159C > T	p.R387*	-	-	DC	-	77	A	P *(3.657×10^{-5})		HT mother	
F-1	E1β	Ex-3	c.301G > A	p.G101S	PD	D	DC	Dec	100	A	A	ITM	1 CHT sibling	This study
		Ex-5	c.581T > G	p.L194R	PD	D	DC	Dec	100	A	A			This study
G-1	E1β	Ex-9	c.1016C > T	p.S339L	-	-	-	-	-	-	-	C	HT mother and HT father	[39]
H-1	E1β	Ex-1	c.196G > T	p.G66W	PD	D	DC	Dec	100	A	A	C	HT father	This study
		Ex-9	c.1016C > T	p.S339L	-	-	-	-	-	-	-		HT mother	[39]
I-3	E1β	Ex-3	c.331C > T	p.R111*	-	-	-	-	-	-	-	C	2 HM siblings, HT mother and HT father	[29]
J-1	E1β	Ex-10	c.1094C > G	p.T365R	PD	D	DC	Inc	100	A	A	C	HT father	This study
		Ex-10	c.1159C > T	p.R387*	-	-	DC	-	77	A	P *(3.657×10^{-5})		HT mother	This mutation detected in gnomAD database
K-1	E1β	Ex-2	c.263A > G	p.D88G	PD	D	DC	Dec	88	A	A	C	NA	This study
		Ex-9	c.1016C > T	p.S339L	-	-	-	-	-	-	-			[39]
L-1	E1β	Ex-7	c.781T > C	p.S261P	PD	D	DC	Inc	88	A	A	C	NA	This study
		Ex-7	c.802G > T	p.E268*	-	-	DC	-	77	A	A			This study
M-1	E1β	5'UTR-Ex-1	~ 3.3 kb deletion spanning 5'UTR to Exon 1	p.?	-	-	-	-	-	-	-	C	NA	[28]
N-1	E1β	5'UTR-Ex-1	~3.3 kb deletion spanning 5'UTR to Exon 1	p.?	-	-	-	-	-	-	-	C	NA	[28]
O-1	E1β	5'UTR-Ex-1	~ 3.3 kb deletion spanning 5'UTR to Exon 1	p.?	-	-	-	-	-	-	-	C	NA	[28]
P-1	E2	Ex-9	c.1196C > G	p.S399C	PD	D	DC	Dec	100	A	A	C	HT mother and HT father	This study
Q-2	E2	Ex-9	c.1066_1067insT	p.V356Cfs*3	-	-	DC	-	NA	A	A	C	1 CHT sibling, HT father	This study
		Ex-11	c.1382C > G	p.S461*	-	-	DC	-	70	A	A		HT mother	This study
R-1	E2	Ex-9	c.1196C > G	p.S399C	PD	D	DC	Dec	100	A	A	C	HT mother and HT father	This study
S-1	E2	Ex-9	c.1196C > G	p.S399C	PD	D	DC	Dec	100	A	A	C	HT mother and HT father	This study
T-1	E2	Ex-9	c.1196C > G	p.S399C	PD	D	DC	Dec	100	A	A	C	HT mother	This study
		Int1	c.51 + 5 G > C	NA	-	-	-	-	-	-	-		HT father	[25]

(*continued on next page*)

Table 2 (*continued*)

Patient	Genetic subtype	Exon/ Intron	Coding DNA number	Protein number	*In-silico* prediction				Screening with 100 normal alleles	Population allele frequency from gnomAD database (Allele Frequency-%)	Clinical phenotype	Co-segregation alleles in family	Reference
					PolyPhen-2.0	SIFT	Mutation taster	I-Mutant	Gene Conservation in Eukaryota (%)				
U-1	E2	Ex-9	c.1196C > G	p.S399C	PD	D	DC	Dec	100	A	A	C	1 HM sibling, HT mother and HT father	This study
V-1	E2	Ex-9	c.1196C > G	p.S399C	PD	D	DC	Dec	100	A	A	C	HT mother and HT father	This study
W-1	E2	Int10-3'UTR Ex-11	4.7-kb deletion flanking parts of intron 10 and the 3'UTR of exon 11	p.?	-	-	-	-	-	-	-	C	HT mother and HT father	[7]
X-1	E2	Ex-9	c.1066_1067insT	p.V356Cfs*3	-	-	DC	-	-	A	A	C	NA	This study
		Ex-9	c.1196C > G	p.S399C	PD	D	DC	Dec	100	A	A			This study
Y-1	E2	Ex-9	c.1196C > G	p.S399C	PD	D	DC	Dec	100	A	A	C	HT mother and HT father	This study

- = not calculable, B = Benign, PD = Probably damaging, D = Damaging, DC = Disease causing, Dec = Decrease, Inc. = Increase, A = Absence; P = Presence, * = This variant is < 1/10000, ITD = Intermediate, C = Classical, ITM = Intermittent, HM = Homozygous, CHT = Compound heterozygous, HT = Heterozygous, NA = Not applicable.

Residue G66 was substituted by the larger and more hydrophobic tryptophan. However, we observed side chain of wild type (G66) and mutant (W66) did not form intramolecular interactions within the protein structure (Fig. 2b). Mutation p.D88G is located on the protein surface. Residue D88 formed hydrogen bond interaction to 4 neighboring residues (S84, T90, A91 and T142), however the substituted G88 exhibited loss of interactions to T90 and T142 (Fig. 2c). Mutation p.G101S is also located on the protein surface. Although both amino acids are classified in similar aliphatic group, neutral and hydrophilic, this substitution could affect the formation of hydrogen bond with surrounding residues (Fig. 2d). We observed the substituted S101 formed an additional interaction with F100. Another new missense mutation, p.L194R is located within loop and interfaces with β, β′ and α′ subunits. We observed the wild type (L194) and mutant (R194) formed hydrogen bond interaction to similar side chain of residue, Q198 (Fig. 2e).

Mutation p.S261P is located within and interfaces with α subunit. The replacement of hydrophilic serine to hydrophobic proline caused loss of hydrogen bond interactions to residues, T306 and P309 (Fig. 2f). Another mutation, p.T273I is located in beta strand. The T273 was observed to form hydrogen bond interactions to 4 neighboring residues (S318, T322, R324 and L326). However, the substituted I273 caused loss of interactions with 2 neighboring residues (S318 and T322) (Fig. 2g). Mutation at position 365 caused a change of threonine to arginine (p.T365R). Residue T365 is located on the C-terminal domain of β and interfaces with α' subunit, and was observed to form hydrogen bond interactions to Y363 and P366. However, the substituted R365 caused loss of hydrogen bond interaction to P366 and at the same time, it formed a new interaction with L413 from α′ subunit (Fig. 2h).

3.2.3. *DBT gene*

The E2b subunits of BCKDC contains an N-terminal lipoyl-bearing domain (LBD), an internal subunit-binding domain (SBD) and a C-terminal core (catalytic) domain (E2bCD) [21,22] . Taking into account the protein sequence of bovine shares 93% identity with human homology and the identified mutation occurs in E2bCD domain, the bovine protein structure was used as a model to study the effects of MSUD mutation in human.

Three new mutations were identified. Nonsense (p.S461*) and frameshift (p.V356CfsX3) mutations found in exons 11 and 9, respectively, were considered to be disease causing due to creation of premature termination codon. New missense mutation p.C399S was found in exon 9. *In silico* analysis predicted this mutation was damaging to the protein function. Structure analysis exhibited both amino acids, serine and cysteine consisted a very similar form of backbone structure but differed in the side chains; serine consisted a hydroxyl side chain while cysteine consisted a sulfhydryl side chain. Side chain of serine formed interaction with a CoA-enzyme ligand, however the substituted C399 was observed to cause loss of interaction to this ligand (Fig. 3).

4. Discussion

To the best of our knowledge, only one mutation in patients with MSUD has been reported in Malaysia [23]. Here, we presented the first comprehensive study of MSUD mutational spectrum for Malaysia population. *T*he present study revealed MSUD was predominant among the Malay ethnic (90.3%). Both *BCKDHB* (45.2%) and *DBT* (38.7%) genes were the most frequent mutated genes in our patient cohort. The finding is in contrary to other studies, in which the most commonly affected genes are being either *BCKDHA* or *BCKDHB* [24–28]. This indicates possibility of *BCKDHB* and *DBT* being the common genes for MSUD in Malaysia population. Therefore, for future molecular screening, especially for Malay ethnic, both genes can be suggested to be proceeded first.

To date, approximately 280 mutations have been reported in HGMD. *BCKDHB* gene has been described as the major gene causing

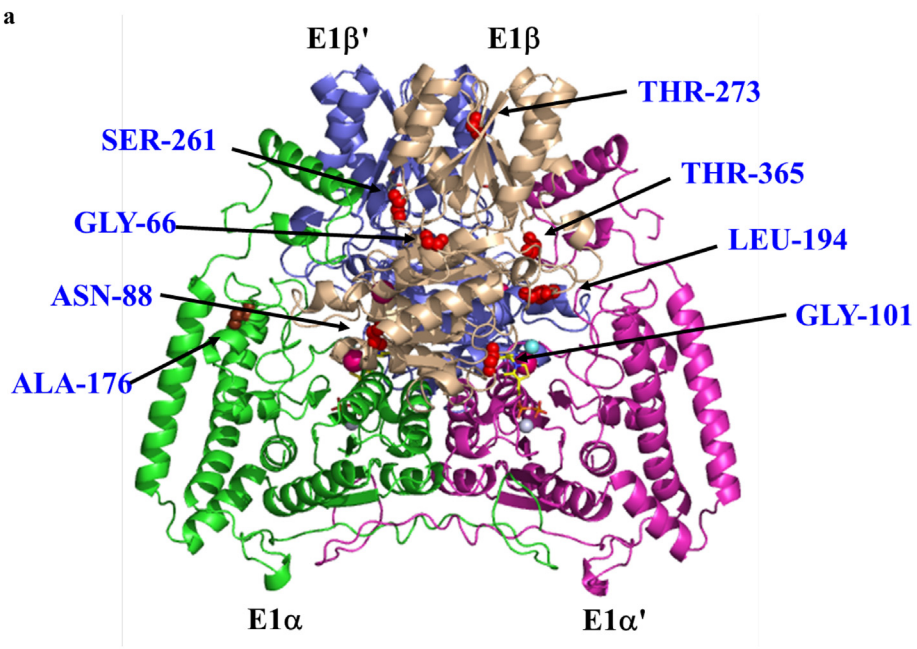

Fig. 1. (a) The predicted 3D-structure of E1β heterotetramer modified from [17] together with the distribution of new missense mutations identified in this study. New missense mutations identified in E1α and E1β subunits are represented by brown and red spheres, respectively. E1α, E1β, E1α' and E1β' are shown in green, wheat, magenta and blue, respectively. The potassium, magnesium and chloride ions are presented in hot-pink, light blue and aquamarine, respectively. Thiamin diphosphates (ThDPs) are shown in yellow stick. The figure was generated with Pymol Version 1.7.4 [20]. (b) The predicted 3D-structure of human E2 subunit model together with the new missense mutation. New missense mutation identified is represented by yellow sphere. E2 subunit is shown in purple and CoA-enzyme in green stick. The figure was generated with Pymol Version 1.7.4 [20]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

MSUD, followed by *BCKDHA* gene and *DBT* gene [8]. In this study, 21 different mutations have been detected and scattered in three genes, confirming the heterogenous distribution of mutations in this disease. 57% of the mutations were accounted to *BCKDHB* gene and the other half was distributed closely between *BCKDHA* (19%) and *DBT* (24%) genes. However, this is contrast with the previous literatures that highlight frequency mutation in *BCKDHA* and *BCKDHB* genes is observed to be equal [24,27,29].

The current study showed 14 out of 21 mutations were identified as new. A new c.1196C > G (p.S399C) genotype was the most frequent in our samples (16 out of 62 alleles), detected as homozygous and heterozygous in 7 (Patient: P-1, R-1, S-1, U-1 and U-2, V-1, and Y-1) and 2 (Patient: T-1 and X-1) patients, respectively. All patients with this mutation were found to be having MSUD classical phenotype. The p.S399C was predicted to be disease-associated by four *in silico* webservers. In human E2 structure model, residue S399 is corresponding to residue S338 on the bovine E2bCD crystal structure. Residue S338 was located in ligand binding pocket and was found to form hydrogen bond with a peptide nitrogen atom in the pantetheine tail of CoA-enzyme ligand [17]. However, in the present study, substitution from serine to cysteine caused loss of interaction with CoA-enzyme ligand, thus possibly leading to destabilization of the binding pocket of E2 subunit protein structure. In addition, residue S338 was proposed to be involved in the acetyltransfer reaction presumably through stabilization of the tetrahedral intermediate [30]. It is therefore possible this mutation could affect the formation of tetrahedral intermediate interaction and subsequently gives an impact to the catalytic activity of the complex. Meanwhile, the serine residue at this position was highly conserved and therefore changes to cysteine or other amino acids were predicted to be pathogenic.

Currently, founder mutations in *DBT* gene were reported in Filipino population (a 239-bp insertion after exon 10) [31] and in general population of Paiwan Austronesian Aboriginal tribe in southern Taiwan (4.7-kb deletion of intron 10) [7]. Founder mutations are also remarked in *BCKDHA* and *BCKDHB* genes and identified in 3 different populations; (1) c.1312 T > A (p.Y438N) found in *BCKDHA* gene is associated in Mennonite populations in US [32], (2) Portuguese Gypsy has been reported to harbor c.117delC (p.R40GfsX23) in *BCKDHA* gene [33] and (3) c. 538G > C (p.R183P) mutation in *BCKDHB* gene is discovered in Ashkenazi Jewish population [34]. In the current study, this new missense mutation p.S399C was predominantly observed in Malay ethnic. However, we were unable to run haplotype analysis to justify this mutation as founder effect in our population, but we have identified this mutation was absent in 100 normal control alleles and also in

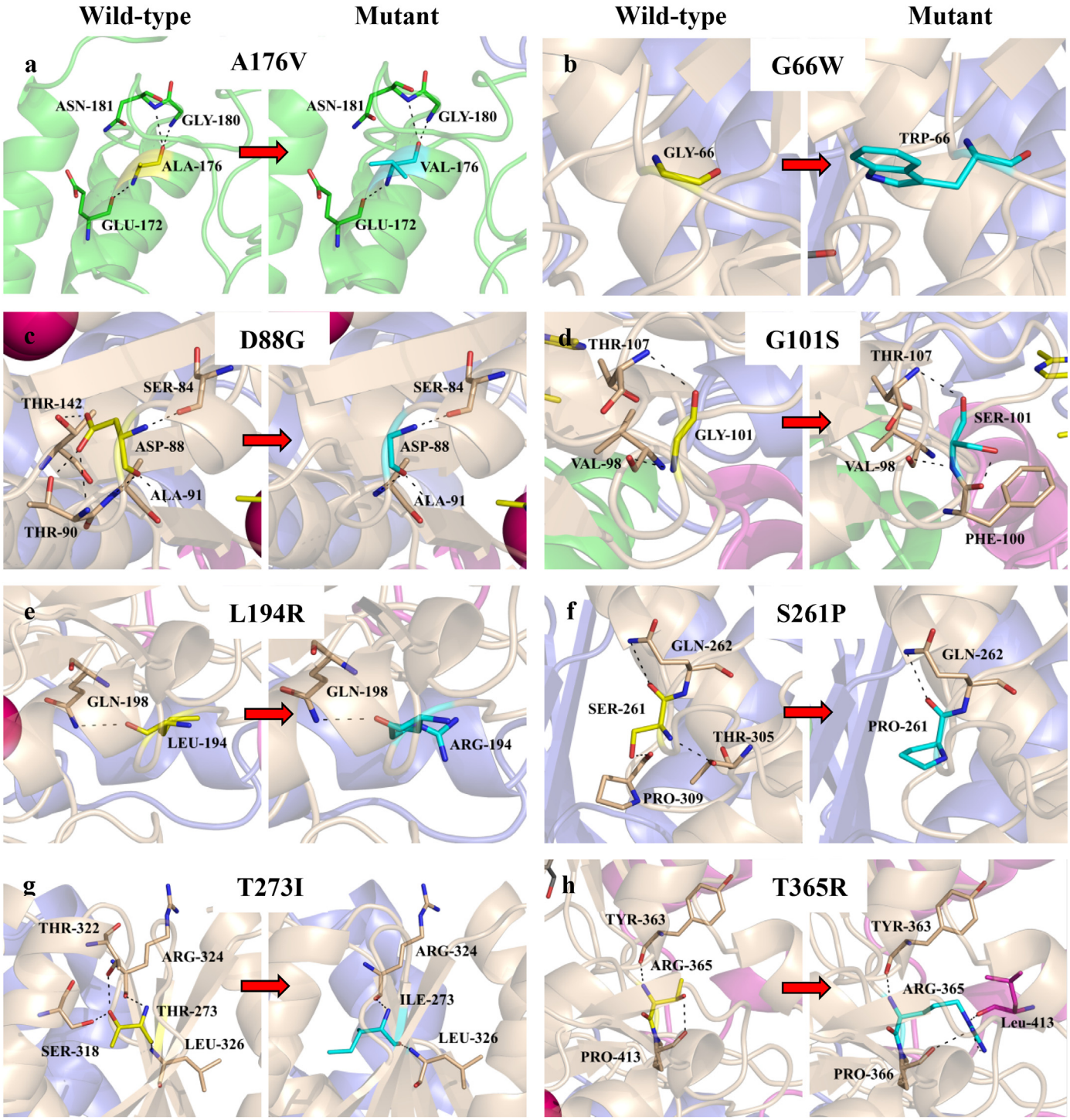

Fig. 2. Structural illustration of the new missense mutations in E1b heterotetramer. a) New missense mutation in α subunit. b-h) New missense mutations in β subunit. Wild-type residue is in yellow sticks while mutated residue is in cyan sticks. Hydrogen bonds are denoted by black dotted lines. E1α, E1β, E1α' and E1β' are shown in green, wheat, magenta and blue, respectively. The potassium, magnesium and chloride ions are presented in hotpink, light blue and aquamarine, respectively. Thiamin diphosphates (ThDPs) are shown in yellow stick. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

gnomAD database. Therefore, we assumed this mutation would be a common and unique to Malay community in Malaysia population.

The heterozygous genotypes for new missense mutations in *BCKDHB* gene; c.301G > A (p.G101S), c.581 T > G (p.L194R), c.196G > T (p.G66W), c.1094C > G (p.T365R), c.263A > G (p.D88G) and c.781T > C (p.S261P) were suggested as very rare variant in general population as it was not detected in our 100 normal control alleles and also in gnomAD database. For another missense mutation, c.818C > T (p.T273I), although it was found in gnomAD database, but it was not detected in homozygous state and the alleles frequency was very low (MAF < 1/10,000). Furthermore, a homozygous missense mutation at the same position (p.T273P) has previously been reported in Saudi Arabia patient with intermediate phenotype [35]. Therefore, this variant can also be considered as a rare in the general population.

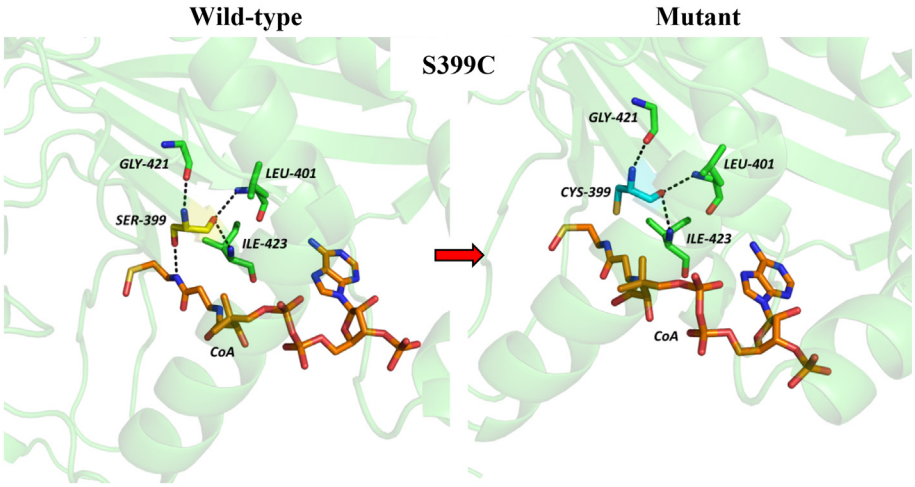

Fig. 3. Structural analysis for new missense mutation in human E2 subunit model. The E2 subunit is shown in green transparency cartoon. Wild-type residue is in yellow sticks while mutated residue is in cyan sticks. CoA-enzyme (CoA) is shown in orange stick. Hydrogen bonds are denoted by black dotted lines. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Structure observation showed missense mutations, p.T273I, p.G101S, p.G66W, p.T365R, p.D88G and p.S261P were possibly leading to destabilization of E1b structure [3]. For mutation p.L194R, although did not affect the surrounding hydrogen bond interaction but it was discovered as compound heterozygous which formed along with new missense mutation, p.G101S. The p.G101S was observed to cause an effect to the E1b protein structure due to formation of extra hydrogen bond with the surrounding residue. Therefore, both mutations were predicted to indirectly affect the E1b structure. Furthermore, both mutations segregated in family F, where 2 patients were affected (Patient F-1 and Patient F-2) with a compound heterozygous, while their parents were heterozygous. Further analysis by *in silico* servers predicted all missense mutations in *BCKDHB* gene were pathogenic. Meanwhile, these mutations affect the highly conserved residue, further confirming pathogenicity. Clinical presentations in all affected patients were an evidence, which all patients suffered from classic and intermittent forms of the disease.

A homozygous genotype for a new missense mutation c.527 C > T (p.A176V) in *BCKDHA* gene was found in family B. Although one *in silico* server predicted this new mutation as benign, however, this mutation segregated in this family, in which 2 patients were affected with homozygous genotype. Both affected patients had MSUD intermediate phenotype. In addition, this mutation was not found in HGMD and 1000G project databases. This mutation was also not detected in our 100 normal control alleles and in gnomAD database, suggesting this mutation was very rare in general population. Another new homozygous genotype in *BCKDHA* gene was detected as splice site c.108 + 6 T > C. However, the pathogenicity of this mutation could not be validated due to no RNA sample. Nevertheless, analysis by *in silico* server to this intronic variant was predicted to eliminate the respective donor site. It is expected that this mutation would produce unstable mRNA and hence leading to production of a protein with null effect [36,37]. Furthermore, this mutation was not presented in our 100 normal control alleles and also not discovered in gnomAD database, suggesting this mutation was very rare in general population. Clinical presentation in Patient-D1 was an evidence, in which he was suffered from classic form of the disease.

Three heterozygous genotypes for new nonsense mutations were discovered; 2 in *BCKDHB* gene, c.802G > T (p.E268*) and c.1159C > T (p.R387*) and 1 in *DBT* gene c.1382C > G (p.S461*). In addition, one new frame shift mutation was found in *DBT* gene c.1066_1067insT (p.V356Cfs*3). The p.E268*, p.S461* and p.V356Cfs*3 were suggested very rare variants in general population as those variants were not presented in our 100 normal control alleles and also in gnomAD database. The p.R387* was discovered in gnoMAD database, however the alleles frequency was very low when compared to the world-wide incidence rate. In addition, this mutation was not found in homozygous state, implying this mutation was a rare in the general population. The nonsense and frame shift mutations were predicted to be pathogenic as those mutations resulted to code for stop codon in the transcribed mRNA and possibly caused an effect in formation of the normal protein function. Furthermore, all nonsense and frame shift mutations were predicted to be disease causing by *in silico* server, further confirming the pathogenicity. The effects of these mutations were consistent with the clinical presentation in all patients, in which the patients have MSUD classical phenotype.

5. Conclusion

In conclusion, 21 different mutations were identified in *BCKDHA*, *BCKDHB* and *DBT* genes from 31 patients. Fourteen were new mutations and scattered along the E1α, E1β, and E2 subunits. All new mutations were predicted to potentially destabilize formation of the E1 and E2 complexes. Interestingly, mutation in *DBT* gene, c.1196C > G (S399C) was discovered to be as recurrent mutation and commonly found in Malay community. Therefore, our study expands the mutation spectrum of MSUD and finding of recurrent mutation is important for disease management and genetic counseling for Malaysia population.

Conflicts of interest

All the authors declared that they have no conflicts of interest to this work.

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References

[1] D.T. Chuang, V.E. Shih, Maple syrup urine disease (branched-chain ketoaciduria), in: C.R. Sciver, A.L. Beaudet, D. Valle (Eds.), The Metabolic and Molecular Basis of Inherited Disease, McGraw-Hill, New York, USA, 2001.

[2] L.J. Reed, Z. Daminu, M.L. Merryfield, Regulation of mammalian pyruvate and branched-chain α-keto acid dehydrogenase complexes by phosphorylation — dephosphorylation, Curr. Top. Cell. Regul. 27 (1985) 41–49, https://doi.org/10.

1016/B978-0-12-152827-0.50011-6.
[3] A. Aevarsson, J.L. Chuang, R.M. Wynn, S. Turley, D.T. Chuang, W.G. Hol, Crystal structure of human branched-chain α α-ketoacid dehydrogenase and the molecular basis of multienzyme complex deficiency in maple syrup urine disease, Structure 8 (2000) 277–291.
[4] Z.M. Yunus, D.A. Kamaludin, M. Mamat, Y.S. Choy, L. Ngu, Clinical and biochemical profiles of maple syrup urine disease in malaysian children, JIMD Rep. 5 (2012) 99–107, https://doi.org/10.1007/8904_2011_105.
[5] P. Rodríguez-Pombo, R. Navarrete, B. Merinero, P. Gómez-Puertas, M. Ugarte, Mutational spectrum of maple syrup urine disease in Spain, Hum. Mutat. 27 (2006) 715, https://doi.org/10.1002/humu.9428.
[6] R.H. Don, P.T. Cox, B.J. Wainwright, K. Baker, J.S. Mattick, "Touchdown" PCR to circumvent spurious priming during gene amplification, Nucleic Acids Res. 19 (1991) 4008.
[7] C.S. Chi, C.R. Tsai, L.H. Chen, H.F. Lee, B. Suk, Chun Mak, S.H. Yang, T.Y. Wang, S.G. Shu, C.H. Chen, Maple syrup urine disease in the Austronesian aboriginal tribe Paiwan of Taiwan: a novel DBT (E2) gene 4.7 kb founder deletion caused by a nonhomologous recombination between LINE-1 and Alu and the carrier-frequency determination, Eur. J. Hum. Genet. 11 (2003) 931–936, https://doi.org/10.1038/sj.ejhg.5201069.
[8] P.D. Stenson, M. Mort, E.V. Ball, K. Evans, M. Hayden, S. Heywood, et al., The Human Gene Mutation Database: towards a comprehensive repository of inherited mutation data for medical research, genetic diagnosis and next-generation sequencing studies, Hum. Genet. 136 (2017) 665–677, https://doi.org/10.1007/s00439-017-1779-6.
[9] The 1000 Genomes Project Consortium, A global reference for human genetic variation, Nature 526 (2015) 68–74, https://doi.org/10.1038/nature15393.
[10] M. Lek, K.J. Karczewski, E.V. Minikel, K.E. Samocha, E. Banks, T. Fennell, H.O. Anne, J. Ware, andrew J. Hill, B.B. Cummings, T. Tukiainen, D.P. Birnbaum, J. Kosmicki, L.E. Duncan, K. Estrada, F. Zhao, J. Zou, E. Pierce-Hoffman, J. Berghout, et al., Analysis of protein-coding genetic variation in 60,706 humans, Nat. Publ. Gr. 536 (2016) 57, , https://doi.org/10.1038/nature19057.
[11] I.A. Adzhubei, S. Schmidt, L. Peshkin, V.E. Ramensky, A. Gerasimova, P. Bork, A.S. Kondrashov, S.R. Sunyaev, A method and server for predicting damaging missense mutations, Nat. Methods 7 (2010) 248–249, https://doi.org/10.1038/nmeth0410-248.
[12] P. Kumar, S. Henikoff, P.C. Ng, Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm, Nat. Protoc. 4 (2009) 1073–1081, https://doi.org/10.1038/nprot.2009.86.
[13] J.M. Schwarz, D.N. Cooper, M. Schuelke, D. Seelow, MutationTaster2: mutation prediction for the deep-sequencing age, Nat. Methods 11 (2014) 361–362, https://doi.org/10.1038/nmeth.2890.
[14] E. Capriotti, P. Fariselli, R. Casadio, I-Mutant2.0: predicting stability changes upon mutation from the protein sequence or structure, Nucleic Acids Res. 33 (2005) 306–310, https://doi.org/10.1093/nar/gki375.
[15] P.W. Rose, A. Prlí C, C. Bi, W.F. Bluhm, C.H. Christie, S. Dutta, R. Kramer Green, D. S. Goodsell, J.D. Westbrook, J. Woo, J. Young, C. Zardecki, H.M. Berman, P.E. Bourne, S.K. Burley, The RCSB Protein Data Bank: views of structural biology for basic and applied research and education, Nucleic Acids Res. 43 (2015). doi:https://doi.org/10.1093/nar/gku1214
[16] R.M. Wynn, M. Kato, M. Machius, J.L. Chuang, J. Li, D.R. Tomchick, D.T. Chuang, Molecular Mechanism for Regulation of the Human Mitochondrial Branched-Chain α-Ketoacid Dehydrogenase complex by Phosphorylation, Structure 12 (2004) 2185–2196, https://doi.org/10.1016/j.str.2004.09.013.
[17] M. Kato, R.M. Wynn, J.L. Chuang, C.A. Brautigam, M. Custorio, D.T. Chuang, A synchronized substrate-gating mechanism revealed by cubic-core structure of the bovine branched-chain a-ketoacid dehydrogenase complex, EMBO J. 25 (2006) 5983–5994.
[18] M.A. Martí-Renom, A.C. Stuart, A. Fiser, R. Sánchez, F. Melo, A. Šali, Comparative Protein Structure Modeling of Genes and Genomes, Annu. Rev. Biophys. Biomol. Struct. 29 (2000) 291–325, https://doi.org/10.1146/annurev.biophys.29.1.291.
[19] B. Webb, A. Sali, Comparative protein structure modeling using MODELLER, Curr. Protoc. Bioinformatics 54 (2017), https://doi.org/10.1002/cpbi.3.
[20] W.L. Delano, The PyMOL molecular graphics system, DeLano Sci. San Carlos, CA 30 (2002) 442–454.
[21] T.A. Griffin, K.S. Lau, D.T. Chuang, Characterization and Conservation of the Inner E2 Core Domain Structure of Branched-chain &-Keto Acid Dehydrogenase complex from Bovine Liver, J. Biol. Chem. 263 (1988) 14008–14014.
[22] K.S. Lau, J. Lee, C.W. Fisher, R.P. Cox, D.T. Chuang, Premature termination of transcription and alternative splicing in the human transacylase (E2) gene of the branched-chain alpha-ketoacid dehydrogenase complex, FEBS Lett. 279 (1991) 229–232.
[23] E.Z. Ali, Z.M. Yunus, N.M. Desa, N.L. Hock, Identification of a novel homozygous mutation (S144I) in a Malay patient with maple syrup urine disease, J. Pediatr. Endocrinol. Metab. 26 (2013) 975–980, https://doi.org/10.1515/jpem-2012-0424.
[24] M.M. Nellis, D.J. Danner, Gene Preference in Maple Syrup Urine Disease, Am. J. Hum. Genet. 68 (2001) 232–237, https://doi.org/10.1086/316950.
[25] N. Yang, L. Han, X. Gu, J. Ye, W. Qiu, H. Zhang, Z. Gong, Y. Zhang, Analysis of gene mutations in Chinese patients with maple syrup urine disease, Mol. Genet. Metab. 106 (2012) 412–418, https://doi.org/10.1016/j.ymgme.2012.05.023.
[26] M.D. Bashyam, A.K. Chaudhary, M. Sinha, H.A. Nagarajaram, A.R.R. Devi, L. Bashyam, E.C. Reddy, A. Dalal, Molecular genetic analysis of MSUD from India reveals mutations causing altered protein truncation affecting the C-termini of E1α and E1β, J. Cell. Biochem. 113 (2012) 3122–3132, https://doi.org/10.1002/jcb.24189.
[27] M.P. Narayanan, K.N. Menon, D.M. Vasudevan, Analysis of gene mutations among South Indian patients with maple syrup urine disease: identification of four novel mutations, Indian J. Biochem. Biophys. 50 (2013) 442–446.
[28] D. Gupta, S. Bijarnia-Mahay, R. Saxena, S. Kohli, R. Dua-Puri, J. Verma, E. Thomas, Y. Shigematsu, S. Yamaguchi, R. Deb, I.C. Verma, Identification of mutations, genotype-phenotype correlation and prenatal diagnosis of maple syrup urine disease in Indian patients, Eur. J. Med. Genet. 58 (2015) 471–478, https://doi.org/10.1016/j.ejmg.2015.08.002.
[29] N. Flaschker, O. Feyen, S. Fend, E. Simon, P. Schadewaldt, U. Wendel, Description of the mutations in 15 subjects with variant forms of maple syrup urine disease, J. Inherit. Metab. Dis. 30 (2007) 903–909, https://doi.org/10.1007/s10545-007-0579-x.
[30] M. Meng, D.T. Chuang, Site-directed mutagenesis and functional analysis of the active-site residues of the E2 component of bovine branched-chain alpha-keto acid dehydrogenase complex, Biochemistry 33 (1994) 12879–12885.
[31] C.L.T. Silao, C.D. Padilla, M. Matsuo, A novel deletion creating a new terminal exon of the dihydrolipoyl transacylase gene is a founder mutation of Filipino maple syrup urine disease, Mol. Genet. Metab. 81 (2004) 100–104, https://doi.org/10.1016/J.YMGME.2003.10.006.
[32] E.G. Puffenberger, Genetic heritage of the Old Order Mennonites of southeastern Pennsylvania, Am. J. Med. Genet. 121C (2003) 18–31, https://doi.org/10.1002/ajmg.c.20003.
[33] S. Quental, A. Gusmão, P. Rodríguez-Pombo, M. Ugarte, L. Vilarinho, A. Amorim, M.J. Prata, Revisiting MSUD in Portuguese Gypsies: evidence for a Founder Mutation and for a Mutational Hotspot within the *BCKDHA* Gene, Ann. Hum. Genet. 73 (2009) 298–303, https://doi.org/10.1111/j.1469-1809.2009.00518.x.
[34] L. Edelmann, M.P. Wasserstein, R. Kornreich, C. Sansaricq, S.E. Snyderman, G.A. Diaz, Report Maple Syrup Urine Disease: Identification and Carrier-Frequency Determination of a Novel Founder Mutation in the Ashkenazi Jewish Population, Am. J. Hum. Genet. 69 (2001) 863–868.
[35] F. Imtiaz, A. Al-Mostafa, R. Allam, K. Ramzan, N. Al-Tassan, A.I. Tahir, N.S. Al-Numair, M.H. Al-Hamed, Z. Al-Hassnan, M. Al-Owain, H. Al-Zaidan, M. Al-Amoudi, A. Qari, A. Balobaid, M. Al-Sayed, Twenty novel mutations in BCKDHA, BCKDHB and DBT genes in a cohort of 52 Saudi Arabian patients with maple syrup urine disease, Mol. Genet. Metab. Rep. 11 (11) (2017) 17–23, https://doi.org/10.1016/j.ymgmr.2017.03.006.
[36] S. Richards, N. Aziz, S. Bale, D. Bick, S. Das, J. Gastier-Foster, W.W. Grody, M. Hegde, E. Lyon, E. Spector, K. Voelkerding, H.L. Rehm, Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology, Genet. Med. 17 (2015) 405–424, https://doi.org/10.1038/gim.2015.30.
[37] K. Nykamp, M. Anderson, M. Powers, J. Garcia, B. Herrera, Y.-Y. Ho, Y. Kobayashi, N. Patil, J. Thusberg, M. Westbrook, S. Topper, Sherloc: a comprehensive refinement of the ACMG-AMP variant classification criteria, Nat. Publ. Gr. 19 (2017) 1105–1117, https://doi.org/10.1038/gim.2017.37.
[38] D. Danner, C. Doering, Human mutations affecting branched chain alpha-ketoacid dehydrogenase, Front. Biosci. 3 (1998) 517–524.
[39] R.M. Wynn, J.L. Chuang, C. Sansaricqţ, H. Mandel, D.T. Chuang, Biochemical Basis of Type IB (E1β) Mutations in Maple Syrup Urine Disease a PREVALENT ALLELE IN PATIENTS FROM THE DRUZE KINDRED IN ISRAEL*, J. Biol. Chem. 276 (2001) 36550–36556, https://doi.org/10.1074/jbc.M105862200.