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Twenty novel mutations in *BCKDHA*, *BCKDHB* and *DBT* genes in a cohort of 52 Saudi Arabian patients with maple syrup urine disease

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ABSTRACT

Maple syrup urine disease (MSUD), an autosomal recessive inborn error of metabolism due to defects in the branched-chain α-ketoacid dehydrogenase (BCKD) complex, is commonly observed among other inherited metabolic disorders in the kingdom of Saudi Arabia. This report presents the results of mutation analysis of three of the four genes encoding the BCKD complex in 52 biochemically diagnosed MSUD patients originating from Saudi Arabia. The 25 mutations (20 novel) detected spanned across the entire coding regions of the *BCKHDA*, *BCKDHB* and *DBT* genes. There were no mutations found in the *DLD* gene in this cohort of patients. Prediction effects, conservation and modelling of novel mutations demonstrated that all were predicted to be disease-causing. All mutations presented in a homozygous form and we did not detect the presence of a "founder" mutation in any of three genes. In addition, prenatal molecular genetic testing was successfully carried out on chorionic villus samples or amniocenteses in 10 expectant mothers with affected children with MSUD, molecularly characterized by this study.

1. Introduction

Maple syrup urine disease (MSUD, OMIM# 248600) is an autosomal recessive inborn error of metabolism. The disease is caused by pathogenic mutations in four genes that encode the three subunits of the mitochondrial complex branched-chain alpha keto acid dehydrogenase (BCKD). These are *BCKDHA* gene encoding for E1 α subunit, (types Ia [OMIM 608348]), *BCKDHB* gene encoding for EI β subunit (type Ib [OMIM 248611]), *DBT* gene encoding for E2 subunit (type II [OMIM 248610]) and *DLD* gene encoding for the E3 subunit of the BCKD complex, the pyruvate dehydrogenase (PD) complex and the alpha-ketoglutarate dehydrogenase (KGD) complex. Mutation in *DLD* does not lead to MSUD, but to dihydrolipoamide dehydrogenase deficiency (OMIM #246900) a combined deficiency of the BCKD, PD and KGD complexes [1]. BCKD catalyzes oxidative decarboxylation of branched-chain α-keto acids and its deficiency results in accumulation of branched chain amino acids (BCAAs; leucine, isoleucine and valine) and their respective keto acids. These compounds especially leucine are neurotoxic and uncontrolled disease results in progressive neurodegenerative course [2,3].

There are four clinical patterns that are observed for MSUD with decreasing severity; a classical phenotype which typically manifests with neurotoxic symptoms in the neonatal period soon after protein intake, an intermediate phenotype that appears later in infancy or childhood, an intermittent phenotype and lastly thiamine-responsive MSUD [2]. There is good correlation with BCKD enzyme activity for the first three phenotypes with most reduced activity observed in classic MSUD. Thiamine pyrophosphate is a cofactor for the E1 subunit and thiamine-responsive MSUD is rare but responds to large doses of thiamine by increasing the catalytic activity of BCKD complex [1,4].

MSUD affects all ethnic groups and has an estimated worldwide frequency of 1/185,000 [4]. It is much more commonly seen in the Pennsylvania old order Mennonite population due to a founder effect (incidence as high as 1/176 reported) in this population [5]. A founder mutation is also reported in Ashkenazi Jews [6] and in a Portuguese gypsy cohort [7]. The exact incidence of MSUD among live births in Saudi Arabia is not known, however newborn screening results suggest that this disease has an estimated frequency of 1 in every 21,490 live

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newborns (unpublished data). This is considered high when compared to worldwide frequency, but is not surprising due to the high rate of consanguineous marriages in Saudi Arabia.

At present, according to the Human Gene Mutation Database (HGMD; http://www.hgmd.cf.ac.uk/ac/index.php) 259 mutations causing MSUD have been reported. In this study we found 25 *BCKDHA*, *BCKDHB* and *DBT* gene mutations (20 novel) in 52 biochemically diagnosed MSUD patients from Saudi Arabia.

2. Patients and methods

2.1. Patients

This study includes samples from 52 patients from a total of 39 different nuclear families diagnosed with MSUD at King Faisal Specialist Hospital & Research Centre (KFSH&RC), Riyadh, Saudi Arabia. All of the patients described (where clinical information was available) were born to consanguineous parents. The patients were either identified on admission to the hospital presenting with clinical symptoms of the classic form of MSUD or biochemically as routine newborn metabolic disease bloodspot testing at the National Laboratory for Newborn Screening on dried blood spots (DBS) located at KFSH&RC. Sample collection adhered to institutional guidelines and to the tenets of the Declaration of Helsinki.

2.2. Biochemical studies

The biochemical diagnosis in MSUD patients was confirmed in DBS that shows a high level of amino acids mainly the branched-chain amino acids [8] by liquid chromatography tandem mass spectrometry (LC-MS/MS; Waters Corporation, USA). Gas chromatography mass spectrometry (GCMS) using HP-5890 interfaced with a model HP-5970 mass spectrometer (Agilent Technologies, USA) was used to analyze urinary organic acids based on the study by Fu and colleagues [9]. In addition, plasma amino acids were analyzed in suspected MSUD patients (Biochrom, Cambridge, UK).

2.3. Mutation analysis in BCKDHA, BCKDHB, DBT and DLD genes

Whole venous blood samples (5–10 ml) from all the patients for molecular genetic analysis were obtained from clinically and biochemically diagnosed patients with MSUD and their parents (if applicable). Genomic DNA extraction was performed using the PUREGENE DNA Extraction Kit according to the manufacturer's instructions (Gentra Systems, Minneapolis, MN).

Genomic DNA of all individuals was amplified by PCR using intronic primers that were designed using the UCSC Genome Browser (http://genome.ucsc.edu/cgi-bin/hgGateway) Exon Primer program to flank each of the coding exons of *BCKDHA* (9 exons), *BCKDHB* (11 exons) *DBT* (11 exons) and *DLD* (14 exons). Primer sequences and PCR conditions are available on request. PCR products were desalted and unincorporated nucleotides removed using ethanol precipitation and directly sequenced using the dideoxy chain-termination method using an ABI Prism Big Dye Terminator v3.1 Cycle Sequencing Kit following the manufacturer's instructions for processing on the ABI Prism 3730 Genetic Analyzer (Applied Biosystems, Inc., Foster City, CA, USA). Purified PCR products covering the entire coding region of each of the three genes identified on Ensembl (http://www.ensembl.org/index.html). Sequence analysis of the gene specific PCR products was performed using the SeqMan 6.1 module of the Lasergene (DNA Star Inc. WI, USA) software package and them compared to the reference GenBank sequences for each gene: *BCKDHA* accession no: NM_000709.2, *BCKDHB* accession no: NM_000056.2, *DBT* accession no: NM_001918 and *DLD* accession no: NM_001918). Numbering commenced with the A of the ATG initiation codon as +1 for mutation nomenclature purposes as recommended by the Human Genome Variation Society (http://www.hgvs.org/mutnomen/).

2.4. Mutation prediction, protein conservation, and modelling of mutations in BCKDHA, BCKDHB and DBT

Novel mutations were analyzed *in silico* using MutationTaster [10] and Polyphen2 to predict pathogenicity (http://www.mutationtaster.org/, http://genetics.bwh.harvard.edu/pph2/). Protein conservation was performed using the protein sequence alignment program, Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/) using sequences obtained from NCBI BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Intronic variants were analyzed using the BDGP: Splice Site Prediction by Neural Network program (http://www.fruitfly.org/seq_tools/splice.html) and by the NetGene2 (http://www.cbs.dtu.dk/services/NetGene2/) server, both of which are services producing neural network predictions of splice sites in various organisms. 3D protein modelling was performed using MODELLER9v101 [11] for *BCKDHA* and *BCKDHB* protein structures. 1DTW_A (RCSB PDB Code - HUMAN BRANCHED-CHAIN ALPHA-KETO ACID DEHYDROGENASE) was used as a template. *DBT* wild-type and mutant structure were predicted using I-TASSER web server2 [12]. Single Amino Acid Polymorphism data analysis pipeline (SAAPdap; [13]), which contains fourteen structural analyses and one sequence-based analysis [14,15] were used to investigate the local structural effects of the novel missense mutations using Protein Data Bank (PDB) structures (http://www.rcsb.org/pdb/home/home.do).

2.5. Chorionic villus sampling and amniocentesis for molecular prenatal diagnoses

Chorionic villus sampling (CVS) between 10 and 12 weeks and amniocentesis (usually at 15–17 weeks) was performed on 10 expectant mothers who previously had an affected child whose pathogenic mutation was identified by this study (4 in *BCKDHA*, 5 in *BCKDHB* and 1 in *DBT*). Fetal genomic DNA was extracted from either CVS or 20 ml amniotic fluid by the PUREGENE DNA Extraction Kit according to the manufacturer's instructions (Gentra Systems, Minneapolis, MN) and targeted mutation analysis was performed for the specific mutation using PCR and direct Sanger sequencing as described previously.

3. Results

Providing clinical information was available, the patients in this study were diagnosed as either classic or intermediate MSUD, as classified by Strauss and colleagues [2]. However, detailed clinical data was not available on all patients, therefore the general phenotype was presented based on the mutation identified in a particular subset as a whole (Table 1). The majority of the patients described in this study were clinically diagnosed with the “classic” most severe form of MSUD.

Direct sequencing in both the forward and reverse directions in 52 MSUD patients identified a total of 25 different homozygous mutations (Table 1); 9 mutations in *BCKDHA*, 10 mutations in *BCKDHB*, and 6 mutations in the *DBT* gene. Twenty of the mutations were novel and 5 mutations had already previously identified [16–20]. As a whole, 15 mutations were missense, 4 resulted in frameshift, 3 were nonsense and 3 affected splicing. Where parental DNA was available, all of the mutations segregated with the disease phenotype (both parents were heterozygous carriers). All identified mutations spanned across the entire coding regions of all 3 genes and their incidences in our cohort are illustrated in Fig. 1. All of the novel substitution and deletion mutations were not found in 200 ethnically matched normal control samples of genomic DNA that supports that these variants are pathogenic and not population-specific polymorphisms.

Table 1
Homozygous mutations in *BCKDHA*, *BCKDHB* and *DBT* in Saudi Arabian patients with MSUD.

	Mutation (nucleotide)	Protein change	Mutationtaster prediction/score	Polyphen-2.0 prediction/score	Number of patients	Number of families	Clinical phenotype	Reference
BCKDHA	c.347A > G	p.Asp116Gly*	Disease causing (0.99)	Probably damaging (0.997)	1	1	Classic	This study
	c.409G > A	p.Glu137Lys*	Disease causing (0.99)	Probably damaging (1.00)	1	1	N/A	This study
	c.647-1G > C	Splice	—	—	1	1	N/A	Georgiou et al. [16]
	c.660_663delGTAC	p.Y221Qfs*108*	Disease causing (1)	—	1	1	Classic	This study
	c.809G > A	p.Ala270Thr*	Disease causing (0.99)	Probably damaging (1.00)	2	1	Intermediate	This study
	c.896A > C	p.Asp299Ala*	Disease causing (0.99)	Probably damaging (1.00)	1	1	Classic	This study
	c.905A > C	p.Asp302Ala	Disease causing (0.99)	Probably damaging (1.00)	4	3	Classic	Rodriguez-Pombo et al. [17]
	c.940C > T	p.Arg314Ter	Disease causing (1)	—	1	1	Classic	Nellis et al. [18]
	c.1270C > T	p.Gln424Ter*	Disease causing (0.99)	—	1	1	Classic	This study
BCKDHB	c.1A > T	p.Met1?*	Disease causing (1)	—	3	3	Classic	This study
	c.197G > C	p.Gly66Arg*	Disease causing (0.99)	Possibly damaging (0.757)	3	3	Classic	This study
	c.286delGAA	p.E96del*	Disease causing (0.99)	—	1	1	Classic	This study
	c.502C > T	p.Arg168Cys	Disease causing (0.99)	Probably damaging (0.998)	2	2	Classic	Flaschker et al. [19]
	c.574G > A	p.Gly192Arg*	Disease causing (0.99)	Probably damaging (1.00)	1	1	Classic	This study
	c.817A > C	p.Thr273Pro*	Disease causing (0.99)	Probably damaging (1.00)	8	4	Intermediate	This study
	c.853C > T	p.Arg285Ter	Disease causing (1)	—	2	2	N/A	Rodriguez-Pombo et al. [17], Fernandez-Guerra et al. [20]
	c.1004G > A	p.Gly335Asp*	Disease causing (0.99)	Possibly damaging (0.843)	3	2	Classic	This study
	c.1006G > A	p.Gly336Ser*	Disease causing (0.99)	Probably damaging (1.00)	3	3	Classic	This study
	c.1145 T > C	p.Cys382Ser*	Disease causing (0.99)	Possibly damaging (0.905)	1	1	Classic	This study
DBT	c.61delC	p.R21Afs*12*	Disease causing (1)	—	2	1	N/A	This study
	c.74delAT	p.C26Wfs*2*	Disease causing (1)	—	1	1	N/A	This study
	c.137A > G	p.Lys46Arg*	Polymorphism (0.99)	Benign (0.001)	3	1	Classic	This study
	c.939-2A > G	Splice*	—	—	3	1	Classic	This study
	c.1195 T > C	p.Ser399Pro*	Disease causing (0.99)	Probably damaging (1.00)	1	1	Classic	This study
	c.1281 + 3A > G	Splice*	—	—	2	1	N/A	This study

* = novel mutation; N/A = no clinical information available, — = not calculable.

3.1. In silico analyses

Local structural effects of the 10 novel missense mutations were examined and the effects of mutations on protein structure were predicted (Table 2). In addition, all of the 3 intronic variants were predicted to completely abolish the respective donor site according to the online prediction tools used.

3.2. Prenatal diagnoses

Results of CVS and amniocentesis on 10 expectant mothers who previously had an affected child whose pathogenic mutation was identified by this study are shown in Table 3. Eight of the ten fetal samples were determined to be either wild-type normal or heterozygous carrier for the target mutation and therefore the pregnancies were continued. Two of the prenatal tests indicated that 2 of the fetuses were homozygous for two separate mutations in *BCKDHB* (c.1A > T and c.574G > A). In these cases, where the clinical phenotype of previous patients demonstrated the "classic" severe form of MSUD, both mothers opted for termination of pregnancy.

4. Discussion

In this report we describe the molecular genetic analysis of patients clinically diagnosed with MSUD originating from Saudi Arabia. The 25 mutations identified spanned across three genes known to cause this disease. All mutations presented in a homozygous form, which is expected due to the high level of consanguinity of our patient cohort. We did not identify the presence of a significant "founder" mutation in any of the three genes, suggesting that these mutations have arisen independently. Although the presence of each of the novel missense mutations were excluded from normal controls, the predicted consequence of the amino acid substitutions on the function of the proteins were investigated further using the PolyPhen and Mutation Taster, both tools which predict the possible impact of an amino acid substitution on the structure and function of a human protein using various structural and statistical parameters. 19 of the 20 novel mutations were predicted to be possibly/probably damaging or disease causing after analysis with PolyPhen and MutationTaster, respectively. The (p.Lys46Arg) mutation in the *DBT* gene was the only variation that was predicted to be a polymorphism and benign by MutationTaster and Polyphen, respectively. However, this variation segregated in the

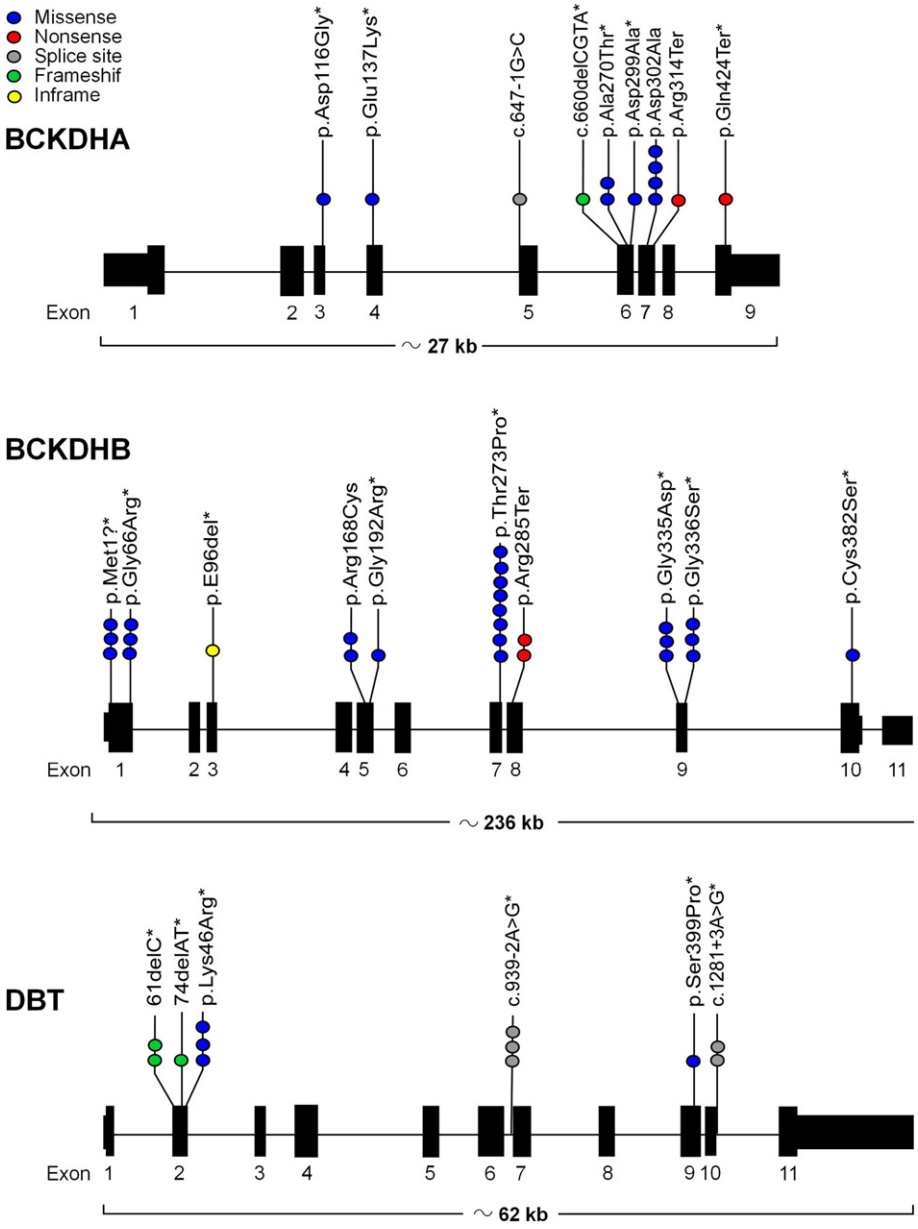

Fig. 1. Schematic representation of known and novel mutations across *BCKDHA*, *BCKDHB*, and *DBT* genes identified in Saudi Arabian patients with MSUD. Novel mutations are highlighted with an asterisk (*) and closed colored circles represent all mutations and their incidence in the cohort of 52 patients.

family (3 affected patients were homozygous, their parents and 2 unaffected siblings were heterozygous), was not seen in 200 ethnically matched normal control samples or found in ExAC (http://exac.broadinstitute.org/) nor the 1000G project (http://www.1000genomes.org/). In addition, CVS prenatal genetic testing at 11 weeks gestational age was performed on the mother of this family specifically targeting the c.137A > G mutation. The fetus was determined to be a heterozygous carrier and did not show any clinical symptoms at her one-year check-up.

With regards to the novel splice site mutations in the *BCKDHA* and *DBT* genes, RNA was not available to validate their pathogenicity on abnormal splicing but were analyzed further *in silico*. All of the 3 intronic variants are predicted to completely abolish the respective donor site according, which is consistent with the classical clinical and biochemical

Notes to Table 2:

[a] Causing an internal void ≥275 Å to open in the protein owing to the substitution with a smaller residue is considered damaging.

[b] Interfaces are defined by a difference in solvent accessibility between a complex and the individual chain in the crystal structure. A difference of >10% is taken as indicative of an interface residue. Interfaces may be with another protein chain or a ligand. Affecting residues in the interface with a different protein chain or ligand more likely reflect biologically relevant interactions by a change in solvent-accessibility.

[c] Hydrophobicity values <0 are hydrophilic.

[d] 99% of sidechains in real proteins have an energy less than 34.33 kcal/mol. Consequently energies >34.33 kcal/mol and <50 kcal/mol can be considered mild clashes, 50–100 kcal/mol medium clashes, >100 kcal/mol severe clashes. Note that clash energies can be extremely high (>>100000 kcal/mol).

[e] A "fully conserved residue" indicates positions which have a single, fully conserved residue. A "highly conserved residue" indicates positions with conservation between groups of strongly similar properties. "Conserved" indicates positions with conservation between groups of weakly similar properties.

profile of this mutation found in the affected patients. Protein sequence alignment (data not shown) of all three MSUD orthologues across a number of species including, human, mouse, and rat, using Clustal Omega, demonstrated that the particular residues of all of the novel missense mutations were highly conserved, further confirming pathogenicity (Table 2).

Table 2
Prediction effects of the novel missense mutations.

Mutation	No. of PDB analyzed	Location of residue	Effect of mutation	Conservation[e]
BCKDHA (P12694)				
p.Asp116Gly	24	α-helix	Instability (destabilizing) -Disruption of hydrogen bond between OD1 of ASP and OH of residue A86 -Introducing a larger void size 294.88 compared to native 244.15[a] -Removal of a buried charge (Native -1; Mutant 0), relative accessibility of native residue: 0.00%	Highly conserved
p.Glu137Lys	26	Coil	Instability (destabilizing) -Disruption of a hydrogen bond between OE1 of GLU and NH1 of residue A220 -Removal of a buried charge (native −1; mutant 1), relative accessibility of native residue: 0.269%	Highly conserved
p.Ala270Thr	24	Coil	Interface-damaging -In the complex, this residue had a relative accessibility of 6.784% while the individual chain had an accessibility of 25.383%, a difference of 18.599%. A difference of >10% is taken as indicative of an interface residue[b]	Fully conserved residue
p.Asp299Ala	25	β-sheet	Instability (destabilizing) -Disruption a hydrogen bond between OD1 of ASP and N of residue A256) -At least 2 structures showed removal of a buried charge (native −1; mutant 0) Interface-damaging -At least two structures showed that native residue was involved in an interface. In the complex, this residue had a relative accessibility of 0.292% while the individual chain had an accessibility of 12.746%, a difference of 12.454%	Fully conserved residue
BCKDHB (P21953)				
p.Gly66Arg	24	Coil	Fold-preventing -The native residue was a glycine and was adopting a backbone conformation not accessible to the other amino acids The native residue was a glycine Native phi angle: 85.696; native psi angle: −168.692 Native pseudo-energy: −1.6075 (0.35 is a threshold above which the energy is considered 'bad') Mutant pseudo-energy: 1.9548 (1.5 is a threshold above which the energy is considered 'bad')	Fully conserved residue
p.Gly192Arg	25	Coil	Instability (destabilizing) -Introduction of a buried charge (native 0; mutant 1) -The mutation introduces a hydrophilic residue into the core of the protein. Native residue hydrophobicity: 0.16 and mutant residue hydrophobicity: −1.8[c] Relative accessibility of native residue: 9.325% Fold-preventing -All structures showed that the replacement sidechain leads to a clash with surrounding residues; the clash energy was 579.44 kcal/mol[d]	Fully conserved residue
p.Thr273Pro	24	β-sheet	Instability (destabilizing) -Disruption of a hydrogen bond between OG1 of the native residue and OG of residue B268) Fold-preventing -All structures showed that the replacement sidechain leads to a clash with surrounding residues. The clash energy was 5547.79 kcal/mol[d] -Proline is introduced at a site where the backbone torsion angles could not accommodate a proline) Native phi angle: −103.648; native psi angle: 133.814 Native pseudo-energy: −3.7006 (1.5 is a threshold above which the energy is considered 'bad') Mutant pseudo-energy: 0.5628 (0.53 is a threshold above which the energy is considered 'bad')	Fully conserved residue
p.Gly335Asp	25	Coil	Instability (destabilizing) Two structures showed that the -Native residue was involved in binding a specific H Bond or van der Waals interaction occurred with another protein or ligand which were disturbed) Interface-damaging -Residue was involved in an interface; in the complex, this residue had a relative accessibility of 23.541% while the individual chain had an accessibility of 72.229%, a difference of 48.688%[b]	Fully conserved residue
p.Gly336Ser	26	Coil	Instability (destabilizing) -Mutation introduces a hydrophilic residue into the core of the protein. Native residue hydrophobicity: 0.16; mutant residue hydrophobicity: −0.26[c] Relative accessibility of native residue: 5.586% Fold preventing -Replacement of sidechain leads to a clash with surrounding residues. The clash energy was 61.69 kcal/mol[c]	Fully conserved residue
p.Cys382Ser	27	α-helix	Instability (destabilizing) -Mutation introduces a hydrophilic residue into the core of the protein. Native residue hydrophobicity: 0.04, mutant residue hydrophobicity: −1.8[c] Relative accessibility of native residue: 0.036% Fold preventing -Replacement of sidechain leads to a clash with surrounding residues. The clash energy was 175.64 kcal/mol.[d]	Fully conserved residue
DBT (P11182)				
p.Lys46Arg			There is no PDB structure to perform the analysis	Conserved
p.Ser399Pro			There is no PDB structure to perform the analysis	Fully conserved residue

Table 3
Results of prenatal genetic testing of targeted mutation analysis of expectant mothers with affected children with MSUD identified by this study.

Fetus	Sample type	Gene	Target mutation	Amino acid change	Genotype status of fetus
1	CVS	*BCKDHA*	c.660_663delGTAC	p.Y221Qfs*108	Heterozygous carrier
2	Amniotic fluid	*BCKDHA*	c.905A > C	p.Asp302Ala	Normal wild-type
3	Amniotic fluid	*BCKDHA*	c.905A > C	p.Asp302Ala	Heterozygous carrier
4	Amniotic fluid	*BCKDHA*	c.905A > C	p.Asp302Ala	Heterozygous carrier
5	CVS	*BCKDHB*	c.1A > T	p.Met1?	Normal wild-type
6	CVS	*BCKDHB*	c.1A > T	p.Met1?	Homozygous affected
7	CVS	*BCKDHB*	c.574G > A	p.Gly192Arg	Homozygous affected
8	CVS	*BCKDHB*	c.817A > C	p.Thr273Pro	Normal wild-type
9	CVS	*BCKDHB*	c.1006G > A	p.Gly336Ser*	Heterozygous carrier
10	CVS	*DBT*	c.137A > G	p.Lys46Arg	Heterozygous carrier

Protein structure prediction was carried out for *BCKDHA*, *BCKDHB* and *DBT* in order to analyze the mutations in a visual way (Fig. 2). In *BCKDHA*, mutation (p.Asp116Gly) is present in the α-helix, thus, affecting the protein helix to some extent with an extra small helix in the following structure. Two mutations (p.Glu137Lys, p.Ala270Thr) are present in coils. As far as, the last mutation is concerned i.e. (p.Asp299Ala), is present in β-sheets, as a result of, extends the immediate β-sheet. Two stop mutations were also identified (p.Arg314Ter, p.Gln424Ter) which led to the impairment of the protein (Fig. 2a). *BCKDHB* structures showed several variations but none of the mutations showed significant change in the structure except p.Thr273Pro. The mutations (p.Arg168Cys, p.Cys382Arg) are present in α-helices and the remaining mutations (p.Gly192Arg, p.Gly335Arg, p.Gly335Asp, p.Gly336Ser) are present in the coils; hence, minimal changes have been discovered in the structure. One stop mutation (p.Arg285Ter) was also detected in the protein which headed to the early truncation of the protein (Fig. 2b). In *DBT* structure, both (p.Lys46Arg, p.Ser399Pro) mutations were present in the coils, thereby, not disturbing the 3D structure (Fig. 2c).

It must be noted that in this report we report the detection of pathogenic mutations in 52 MSUD affected patients, there were however additional patients with classic and intermediate MSUD that a mutation in the coding region in any of the three genes described were not identified, using the primers described. Further molecular analysis will be performed to sequence the promoter, intronic regions and 3'UTR of all three genes specifically in these patients.

The identification of pathogenic mutations causing MSUD will be of tremendous use for molecular diagnosis from patients in Saudi Arabia and for the region in general, with respect to prevention of this disease in the forms of future carrier testing, prenatal testing, pre-marital screening and pre-implantation genetic diagnosis.

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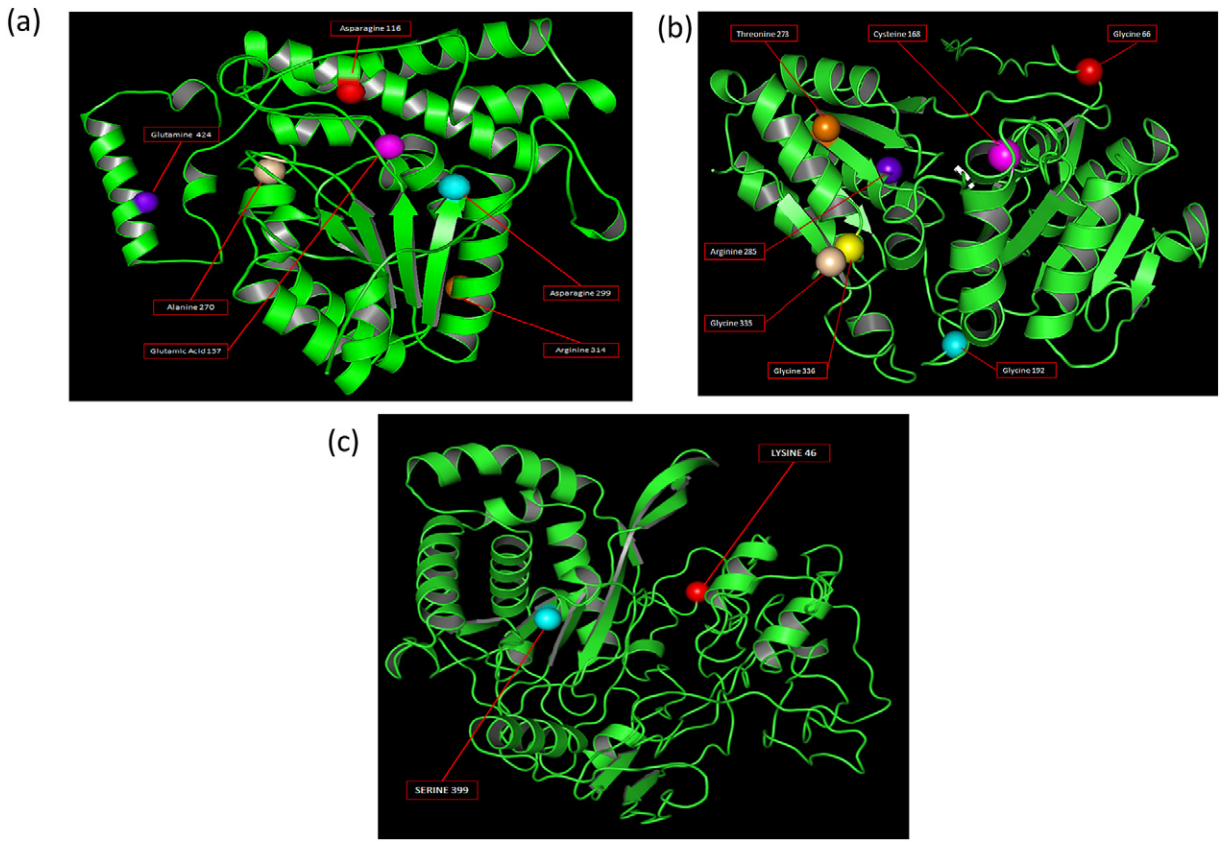

Fig. 2. Normal protein structures where the colored spheres show missense and stop codon mutation locations (a) *BCKDHA* (b) *BCKDHB* and (c) *DBT* mutant residues.

Conflicts of interest

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

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