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The Genes Responsible for Maple Syrup Urine Disease, Molecular Pathomechanisms and Causative Mutations in Iranian Population

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ABSTRACT

BACKGROUND AND OBJECTIVE: Maple syrup urine disease is a rare inborn metabolic inherited disorder caused by deficiency of branched chain α-keto acid dehydrogenase complex and leading to accumulation of branched chain amino acids in body fluid. The incidence of MSUD is higher in populations with high consanguineous marriage. BCKD is a mitochondrial complex which is encoded by four nuclear genes (*BCKDHA*, *BCKDHB*, *DBT*, and *DLD*) and MSUD can be caused by mutation within any of these four genes. Accumulation of metabolic is associated with impairment of energy metabolism, provoke apoptosis, dysfunctional neurotransmitter synthesis and neuropathological defects such as seizure, psychomotor delay and coma. In the present study, we investigated the incidence of MSUD in Iran, compiled previously reported mutations in Iranian population and also explained molecular pathomechanisms underlying MSUD.

METHODS: To compile MSUD mutations, we systematically reviewed PubMed and magiran databases to find related articles in English and Persian language, respectively. The key words "MSUD" and "Iran" was used as query.

FINDINGS: Until 9th December 2018, twenty four MSUD mutations were collected from Iranian population of which 18 mutations have been only identified in Iran and were not reported in other populations yet. Likewise, because of high consanguineous marriages, the incidence of MSUD were higher than worldwide average in different provinces.

CONCLUSION: Identification and compiling of MSUD mutations in Iranian population can be useful for prenatal genetic diagnosis in at risk families and play crucial role in early diagnosis and also treatment before starting neurological symptoms in newborns.

KEY WORDS: *Maple syrup urine disease*, *BCKDHA*, *BCKDHB*, *DBT*, *DLD*, *PPM1K*,MSUD.

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Introduction

Maple syrup urine disease (MSUD; OMIM #248600) is a rare autosomal recessive genetic disorder that is caused by defect in catabolism of the three branched chain amino acids (BCAAs) leucine, isoleucine, valine (1, 2). This disorder was first described by Menkes et.al in Boston children Hospital in 1954 as a familial syndrome in which 4 siblings had progressive infantile cerebral dysfunction associate with an unusual urine (3).

This disease caused by mutation in four genes (BCKDHA, BCKDHB, DBT, DLD) that lead to deficiency of branched chain α keto acid dehydrogenase complex and an accumulation of branched chain amino acids leucine, isoleucine, valine and their corresponding branched chain α keto acids (BCKAs), α-ketoisocaproate (KIC), α-keto-β-methylvalerate (KMV), α-ketoisovalerate (KIV), in tissue and body fluids (1). Of the three, leucine exerts the most influence on cellular function that varies with tissue (4). Accumulation of leucine in particular causes neurological symptoms, whereas elevation of plasma isoleucine is associated with maple syrup urine odor (5). In this paper, the genes responsible for and the molecular pathomechanisms underlying MSUD were reviewed. Likewise, MSUD mutations that already reported in Iranian populations were systematically collected, and also the incidence of MSUD were investigated in different provinces of the country.

The metabolism of Branched chain amino acid: Leucine, isoleucine, valine are three essential amino acids that cannot be synthesized *de novo* in mammals therefore they must be taken in food (6). BCAAs are required for protein synthesis, branched chain fatty acid synthesis, and neurotransmitter synthesis, the catabolism of BCAA must be tightly regulated. The BCKD complex is a mitochondrial multienzyme complex consist of three catalytic components decarboxylase E1, dihydrolipoyl transacylase E2, dihydrolipoamide dehydrogenase E3 and two associated regulatory enzyme, BCKD kinase and BCKD phosphatase (7-9); BCKDC is the most important regulatory enzyme in the catabolic pathways of the BCAAs (Fig. 1) (10).

E1 (two α and two β subunits), E2 and E3 encoded by nuclear BCKDHA, BCKDHB, DBT, DLD genes respectively (1, 11, 12). E3 subunit is shared with pyruvate and α ketoglutarate dehydrogenase complex (13). The initial step in the catabolism of BCAAs is transamination of leu, Ile and Val. The normal degradation of BCAAs begin with transamination by donation of amine group to α-ketoglutarate (α-KG) forming glutamate and their corresponding branched chain keto acid (BCKA). α-ketoisocaproate (KIC), α-keto-β-methylvalerate (KMV), α-ketoisovalerate (KIV) are produced from leucine, isoleucine and valine by transamination. This step is carried out via branched chain amino acid transaminase (BCAT) (Fig2). The second step is the oxidative decarboxylation of the BCKAs by the BCKD complex. The studies have been shown the second step in BCKAA catabolism which is disrupted in MSUD (10, 11, 14).

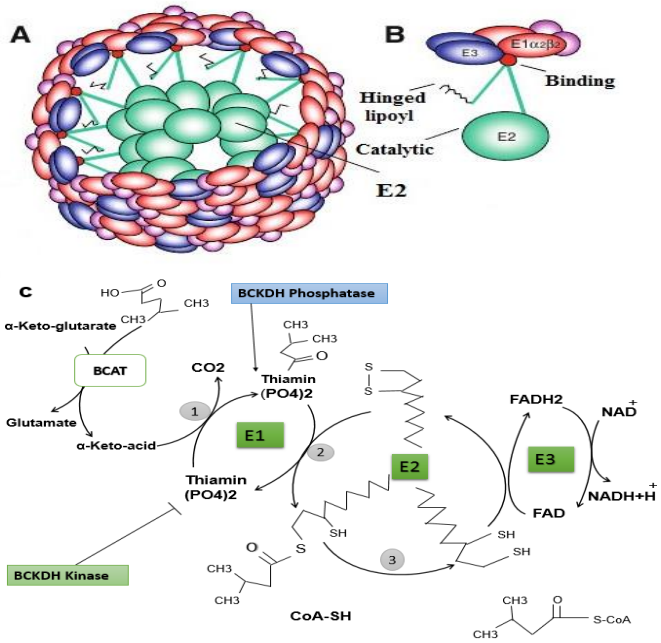

Figure 1. A) The macromolecular structure of branched-chain keto acid dehydrogenase showing a 24-mer E2 cubic core with multiple copies of E1 and E3 forming the outer layer of this enzyme complex. B) Enlarged E1, E2, E3 units showing E3 dimer and E2α2β2 heterotetramer bound to E2 binding domain (red) C) Biochemical reaction diagram, showing leucine transaminated via BCAT to form αKIC. Reaction 1 shows decarboxylation of the α-keto acid. Reaction2, thiamine-diphosphate-dependent acyl-transfer to the lipoyl bound domain of E2, carried out by E1. Reaction 3 involves acyl-transfer to coenzyme A (CoA-SH) by E2.

Regulation of branched-chain α-keto acid dehydrogenase complex: Activity of the branched-chain α-keto acid dehydrogenase complex is controlled by two regulatory enzyme, BCKDH Kinase (BDK,EC 3.1.3.16) and BCKDH phosphatase (BDP,EC 2.7.11). Protein phosphatase BDP (protein phosphatase 2Cm or PP2Cm) is encoded by *PPM1K*. When BCAAs are needed for protein synthesis, BDK phosphorylates E1α

subunit in specific sites (Ser 302, Ser 292) (Fig3) (7, 11). In contrast, dephosphorylation occurs by BDP when BCAAs are present in excess and causing reactivation of the complex (15-17).

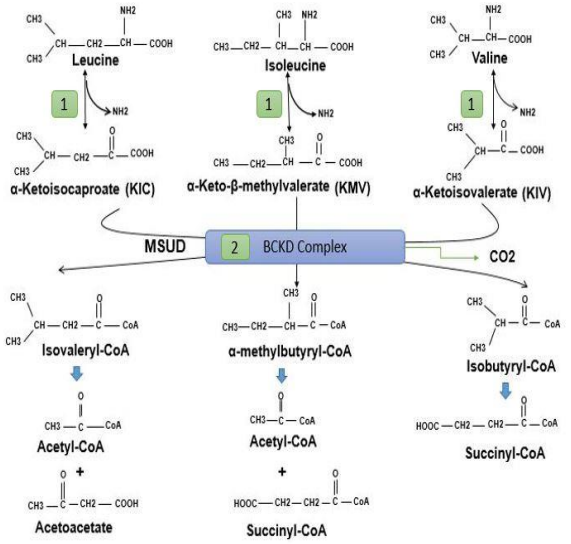

Figure 2. Oxidative degradation of the BCAAs leucine, isoleucine, and valine. The transamination of BCAA is catalyzed by a single branched-chain aminotransferase. α-ketoisocaproate (KIC), α-keto-β-methylvalerate (KMV), α-ketoisovalerate (KIV) are produced from leucine, isoleucine and valine by transamination (reaction 1). The oxidative decarboxylation of BCKAs is catalyzed by the single mitochondrial branched-chain a-ketoacid dehydrogenase complex (reaction 2).

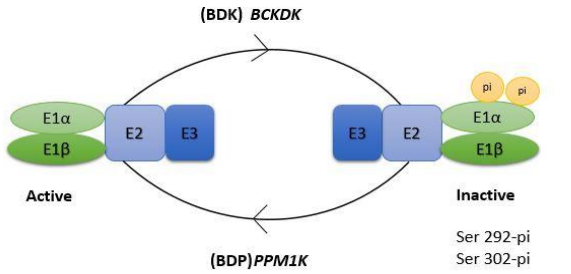

Figure 3. BCKDH complex can be regulated at post transcriptional levels. This enzyme complex phosphorylation by protein kinase BDK causing inactivation. When excess BCKAAs are present, dephosphorylation of E1α by BDP occurs which causing reactivation of complex

Molecular genetics of branched-chain α-keto acid dehydrogenase complex: The first genetic variants linked to MSUD were discovered by Zhang in 1989. The patient was a compound heterozygote in *BCKDHA* (18).The genes involved in MSUD have been listed in table 1. The most mutation in the *BCKDHA* gene or *BCKDHB* occurs with 45% and 35% in patients affected with MSUD (19)

Although *BCKDHA*, *BCKDHB*, *DBT*, *DLD* genes have been identified as the genes involved in this disease in the most of the source, recently *PPM1K* gene has been identified as the fifth gene involved in maple syrup urine disease (Fig4)(20). Therefore, the characteristics of this gene has been investigated separately in the present study; Lu *et at.* found that PP2Cm deficient mice exhibited catabolism of branched chain amino acids and increase in plasma concentration of branched chain amino acids. PP2Cm null mice on a high protein diet showed increased neonatal lethality and increase oxidative stress that also shows metabolic phenotype similar to intermittent and intermediate type of human maple syrup urine disease. Their studies suggest that defects in PP2Cm may be responsible for subset of human MSUD (21).

Oyarzabal *et at.* identified a homozygous mutation (c.417_418delTA) in the *PPM1K* gene in a women with a mild variant of maple syrup urine disease (20). Their finding confirms that defective activation of BCKDH complex, via defect in PP2Cm production, leads to a significant increase in plasma concentrations of BCKA and BCAA that associated with a mild form of MSUD. Analysis of the segregation pattern in the parent showed the father to be heterozygous carrier for the change but (c.417_418delTA) was absent in mother. Segregation analysis of the parent confirms uniparental disomy for chromosome 4. So far, no extra mutation that causes the disease has been reported (20).

Clinical presentation of maple syrup urine disease: MSUD is a heterogeneous disorder with variable clinical presentation severity (17, 24). Based on BCKD activity, the patient affected with MSUD are classified five types. The first form has the most sever clinical manifestation. Activity of BCKDH complex is 0-2% of normal. Affected infants show lethargy, poor feeding, vomiting, and ketoacidosis. The second or intermediate form, the levels of plasma BCAAs and BCKAs are persistently increased. Enzyme activity is 3-40% of normal and present in any age. The third one, intermittent form of MSUD, enzyme activity is 5-20% of normal.

In the intermittent form, the patients have normal development, normal intelligence and normal levels of BCAAs and BCKAs but the symptoms are usually trigged by stress such as infections. The fourth form or thiamine-responsive MSUD is similar to intermediate or

intermittent MSUD and respond to treatment with thiamine lead to normalization of the BCAA levels. The enzyme activity is 2-4%. The fifth or E3 deficiency (dihydrolipoamide dehydrogenase deficiency) and enzyme activity 0-25% of normal (6, 19, 27, 28). The E3 subunit is shared with pyruvate and α-ketoglutarate dehydrogenase complex, decreased activity of this component result in a deficiency in activity of the all enzyme complex presenting variable phenotype.

DLD deficiency associated with Leigh syndrome showed compound heterozgosity for two *DLD* mutations (c.405_407delAGG; c.1058T>C) in a 14 year-old girl (13, 29). E3 deficiency is rare, and involves lactic acidosis (10) as well as elevated BCAAs and BCKAs and fewer than 20 cases reported (30). The classic form, which account for 75% of MSUD patient, is manifested within the first 2 wk of life and death if left untreated (31, 32).

About 25% of patients suffer from variant forms (with a continuum of residual BCKDH activity from 2% to 40%) with later onset (27, 31, 33). Decarboxylation activity range in MSUD types have been presented in table 2.

Table 1. List of genes involved in MSUD

Gene	Cytogenetic location	Transcript number	Polypeptide	Exon	Reference
BCKDHA	19q13.1–13.2	NM_000709.3	445AA	9	(22, 23)
BCKDHB	6q14	NM_000056.4	392AA	11	(24, 25)
DBT	1p21.2	NM_001918.3	482AA	11	(25)
DLD	7q31.1	NM_001289751.1	486AA	14	(26)
PPM1K	4q22.1	NM_152542.4	372 AA	7	(15)

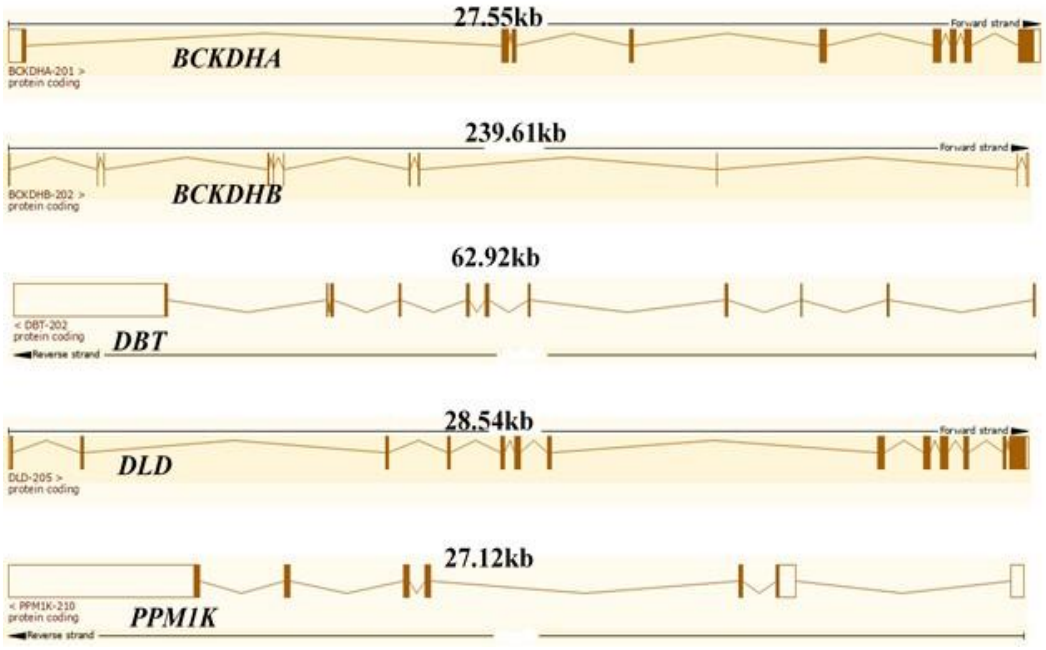

Figure 4. Schematic of exons and introns associated with genes involved in maple syrup urine disease. The blank region represent non-coding parts of an exon. Numbers indicate genes length.(Adapted from Ensembl).

Table 2. Decarboxylation activity rang of α-ketoacid dehydrogenase complex in five MSUD types

Clinical phenotype	Age of onset	Gene	BCKAD subunit	Decarboxylation activity
Classic	Neonatal	*BCKDHA, BCKDHB, DBT*	E1α; E1β; E2	0-2
Intermediate	Variable	*BCKDHA, BCKDHB, DBT*	E1α; E1β; E2	3-30
Intermittent	Variable	*BCKDHA, BCKDHB, DBT*	E1α; E1β; E2	5-20
Thiamine-responsive	Variable	*DBT*	E2	2-40
E3-deficient	Variable	*DLD*	E3	0-25

Oxidative stress and neurological damage in Maple syrup urine disease: Both animal models and human trials investigation demonstrate that deficiency of energy metabolism due to electron transport chain inhibition, neuronal apoptosis, defective and dysfunctional neurotransmitter synthesis, neurotoxicity in MSUD. In the recent year, importance of oxidative stress in pathophysiology of brain damage in MSUD has been attended in patient (34). Free radicals such as free radical species can be generated both in physiological or in pathological condition (35).

The purpose of organism having antioxidant defense system is to control the continuous production of oxidant (35, 36). An imbalance between the production and removal of free radicals can lead to pathological consequence such as stress oxidative (37). Leucine inhibits the antioxidant enzymes and increased oxidative stress(35). Free radicals and oxidative stress are involved in a large number of human disorders like neurological diseases (37, 38). MSUD is a neurological disease that metabolites induce high production of reactive species and deplete antioxidant capacity (35). The exact mechanisms underlying the neurological symptoms of MSUD is not well understood but it is proven that leucine and KIC are the main neurotoxic metabolites in this disorder and the excessive concentration of these metabolites are associated with appearance of neurological symptoms (35).

Leucine and KIC are able to promote DNA damage via increased free radical production (37); likewise, when brain is expose to high concentration of BCAA and the metabolites, it can provoke neuronal apoptosis (39, 40). The increased toxic effect of MSUD metabolites on human fibroblast demonstrate that the cells of these patient are susceptible to apoptosis. This could be due to they lack the enzyme branched chain α keto acid dehydrogenase and not able to metabolize BCKAs (41). Nerve growth factor (NGF) is the prototype number of the neurotrophin protein family that produces in the brain during life and essential for the survival, maturation and maintenance neurons. This growth factor play crucial roles in various signaling pathway that are essential for development, axonal growth and neurotransmission (42, 43).

Free radical production could be involved in decreased NGF levels after the chronic administration of the BCAA in hippocampus; BCAA can be involved in the regulation of NGF in development of adult rat hippocampus. These findings suggest that stress oxidative should be considered as an important pathophysiological mechanism underlying the brain damage in MSUD (43).

The role of cytoskeleton in neuropathology of MSUD: The exact mechanisms related to neurological damage in MSUD are not well understood and need more investigation. Studying animal models demonstrate that the BCKA accumulation in MSUD can alter the cytoskeleton (44). Eukaryotic cytoskeleton mainly consist of microfilaments (MFs), microtubules (MTs) and intermediate filaments (Ifs). IF proteins are classified into five types. Types I and II of IF proteins include the keratins and type III proteins include vimentin, desmin, peripherin and glial fibrillary acidic protein (GFAP), the major IF in mature astrocytes. Type IV of IF proteins, consists of nestin, α-internexin and the neurofilaments (NFs) (44, 45).

Neurofilaments are heteropolymers consist of (NF-l),(NF-M),(NF-H) subunits (46). The cytoskeletal organization in eukaryotic cells depends on the phosphorylation levels of its constituent proteins and activity of the cytoskeletal associated phosphorylation system. Altered protein phosphorylation lead to neurodegeneration (44). Pessoa-Pureur *et al*. studies indicated that accumulation of two metabolites (KIC and KMV) in MSUD provoked significant alteration in NF-H; also the phosphorylation level of NF-H could be increased by KIC, KIV and KMV (47).

BCKA altered phosphorylation of IF proteins in cerebral cortex of rats (48); animal trials also suggest that BCKAs accumulation in MSUD provoke alteration of the IF associated phosphorylation system, morphological change and death in neural cells. BCKA treatment of C6 cells induced an increased polymerization of GFAP phosphorylation, as well as GFAP hyperphosphorylation and disorganization of cellular structure. This could be involved in the brain damage of MSUD patients (45). The abnormal cytoskeletal phosphorylation/ dephosphorylation level induced by the BCKA is deleterious to neural cell function and structure (44, 45).

Methods

To investigate MSUD mutations, we systematically reviewed PubMed and magiran databases to find related articles in both English and Persian languages, respectively on 6th December 2018. To collect these mutations, "Advance search" was used in the PubMed database by applying keywords "MSUD" in (Title/Abstract) combined with "Iran" in (Affiliation).

To compile Persian articles, we checked magiran database (http://www.magiran.com) by applying "MSUD" as keyword in (search).

Results

On the basis of search strategy which mentioned in method section, 20 articles in Persian (magiran) and English (PubMed) were collected in total, of which 6 related articles were selected and reviewed. Finally 24 MSUD mutations were compiled in Iranian population of which 18 mutations have been only identified in Iran and were not reported in other populations yet (Table3). Likewise, because of high consanguineous marriages, the incidence of MSUD were higher than worldwide average in different provinces (Fig5).

The incidence of maple syrup urine disease: The worldwide incidence of MSUD is estimated ranges from 1:185,000 to 1:940,000, however, the prevalence is much higher in certain ethnic groups, specifically the Mennonite populations of Pennsylvania (1:176) (53). Consanguineous marriage in Iran was approximately (38.6%) (54) which it is much higher than worldwide, so autosomal recessive inheritance diseases like MSUD are more prevalent.

Fig 5 shows the incidence rate of MSUD reported in Mazandaran, Tehran and Fars provinces (55-58). The incidence rate of this disease have not been reported in other provinces of the country so far. It is worth to mention that in Isfahan province, during 9 years, among 392 patients affected with inherited metabolic disorders, 18 patient (4.6%) suffered from MSUD, however the incidence of disease has not been mentioned (59).

Table 3. MSUD-causing mutations so far reported in Iranian population

Gene	Mutation at nucleotide level	Mutation at protein level	Reference
BCKDHA	NM_000709.3: c.117delC	p.Arg40GlyfsX23	(49)
BCKDHA	NM_000709.3: c.143delT†	p.Leu48ArgfsX14	(22, 49)
BCKDHA	NM_000709.3: c.288 + 1G > A†	-	(22)
BCKDHA	NM_000709.3: c.[(375 +1 _376-1)_ (884 +1_885-1)del]†	-	(22)
BCKDHA	c.452C > T*	p.Thr151Met	(50)
BCKDHA	c.629C > T*†	p.Ala210Val	(51)
BCKDHA	NM_000709.3: c.702delT†	p.Tyr235ThrfsX94	(22)
BCKDHA	NM_000709.3: c.731G > A†	p.Gly244Glu	(22)
BCKDHA	c.868G > A*	p.Gly290Arg	(51)
BCKDHA	c.890G > A*	p.Arg297His	(51)
BCKDHA	c.938C > A*†	p.Ala313Asp	(50)
BCKDHA	NM_000709.3: c.1167 + 1G > T†	-	(22)
BCKDHA	c.1198delA*†	p.Lys400fsX13	(51)
BCKDHA	c.1267_1267delC*†	p.Gln423fs	(50)
BCKDHB	NM_183050.2: c.[(274 +1_275-1)_(343 +1_344-1)del]†	-	(24)
BCKDHB	c.410C > T*	p.Ala137Val	(51)
BCKDHB	c.496A > G*†	p.Lys166Glu	(50)
BCKDHB	NM_183050.2: c.508G > T†	p.Arg170Cys	(24)
BCKDHB	NM_000056.4: c.508C > T	p.Arg170Cys	(50, 52)
BCKDHB	NM_183050.2: c.633 + 1G > A†	-	(24)
BCKDHB	c.652C >T*†	p.Pro218Ser	(51)
BCKDHB	NM_183050.2: c.833_834insCAC†	p. Gly278_Thr279insThr	(24)
BCKDHB	NM_183050.2: c.988G > A†	p.Glu330lys	(24, 50)
DBT	c.1150A > G*†	p.Ser384Gly	(51)

***The transcript number has not been mentioned in reference article**

† Mutations identified only in Iran and not reported in other populations yet

Figure 5. Incidence rate of MSUD reported in Mazandaran, Tehran and Fars provinces

Discussion

MSUD is a genetically heterogeneous disorder due to mutations in the genes encoding α-ketoacid dehydrogenase. The worldwide incidence of MSUD is estimated to be one in 185,000. Based on the high consanguineous marriage rate in Iran that is much higher than worldwide, therefore more prevalence of this disease is expected. Up to date, more than 200 causative mutations have been identified according to Human Gene Mutation Database (HGMD). In the present article, MSUD mutations in Iranian population have been compiled, of which the majority of mutations (18 out of 24) have been identified in Iran and not reported in other populations yet. Early diagnosis of MSUD play crucial role to prevent disease before neurological symptoms. The compiled MSUD mutations in Iranian population can be useful for at risk families and facilitate early diagnosis and treatment. These mutations can be beneficial for prenatal diagnosis in at risk families and useable for medical genetics laboratories in our country.

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