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A Novel Mutation in the DBT Gene Causes in an Azerbaijanian Child Classic Maple Syrup Urine Disease

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Abstract

Maple syrup urine disease (MSUD) is a complicated disease that is inherited one. The maple syrup urine disease is accompanied with full or partial disorder of enzyme activity, participating in the metabolism of three amino acids as valine, leucine and isoleucine. If the process of valine, leucine and isoleucine metabolism is interrupted, then stockpiling and decay happens in the body. Decay products of those amino acids are evacuated from the body and are toxic. These toxins relate to biogenic amines – ptomaine.

The presence of maple syrup metabolic disorder by biochemical and molecular genetic methods in Azerbaijan population confirms the importance of screening test of newborns for the respective disease.

Homozygous substitution of adenine by guanine mutation has been found in DBT (dihydrolipoamide branched-chain transacylase) gene at 1199 nucleotide position (p. N400S 1199 A (Adenine) - G (Guanine)). No mutations were identified in BCKDHA (branched-chain keto acid dehydrogenase E1, alpha polypeptide) and

BCKDHB (branched-chain keto acid dehydrogenase E1, beta polypeptide) genes. The identified mutation (1199 A (Adenine)-G (Guanine)) is a new mutation and has not been included in any literature. This mutation disrupts the metabolism of Valine, Leucine and Isoleucine amino acids and leads to maple syrup disease.
For the diagnosis of maple syrup metabolic disorder the amount of valine, leucine and isoleucine amino acids was evaluated in the urine and plasma and the comparison of obtained results revealed that plasma analysis was more informative.

Keywords: inherited metabolic disorder, maple syrup metabolic disorder, mutation, neutral genetic polymorphisms

Introduction

Maple syrup urine disease is an inherited disorder in which the body is unable to process certain protein building blocks (amino acids) properly. The condition gets its name from the distinctive sweet odor of affected infants' urine. It is also characterized by poor feeding, vomiting, lack of energy (lethargy), abnormal movements, and delayed development. If untreated, maple syrup urine disease can lead to seizures, coma, and death [1,2].

Maple syrup urine disease is often classified by its pattern of signs and symptoms. The most common and severe form of the disease is the classic type, which becomes apparent soon after birth. Variant forms of the disorder become apparent later in infancy or childhood and are typically milder, but they still lead to delayed development and other health problems if not treated [3,4].

Maple syrup urine disease (MSUD) is a life-threatening metabolic disorder. Metabolic disorders are conditions in which your body can’t function normally because it can’t properly convert food to energy to keep your body healthy [5].

Protein is needed by the body to function normally. Proteins are made up of 20 different types of amino acids. Proteins must be broken down (metabolized) so they can be absorbed and used by the body. People with MSUD don’t have the needed enzymes (either don’t have the specific enzymes at all, have the specific enzymes but they don’t work, or don’t have enough of the specific enzyme) to break down three particular amino acids – leucine, isoleucine and valine.
Because people with MSUD can’t break down these three amino acids, these amino acids build up in the body, become toxic to the body and cause severe health

problems. Without medical management, maple syrup urine disease can lead to a wide range of intellectual and physical disabilities and death [6].

The four main types of MSUD are: 1. Classic: Classic maple syrup urine disease is the most severe type of MSUD. It is also the most common. Symptoms usually develop within the first three days of birth. 2. Intermediate: This type of MSUD is less severe than classic MSUD. Symptoms typically appear in children between the ages of 5 months and 7 years. 3. Intermittent: Children with intermittent MSUD develop as expected until an infection or period of stress cause symptoms to appear. People with intermittent MSUD can usually tolerate higher levels of the three amino acids than people with classic MSUD. 4. Thiamine-responsive: This type of MSUD responds to treatment using high doses of vitamin B1 (thiamine) along with a restricted diet. With treatment, people with thiamine-responsive MSUD have higher tolerance for the three amino acids [7].

MSUD is very rare. It occurs in about 1 of every 185,000 births worldwide. It appears more often in populations with a small gene pool or when cousins and other close relatives have children together. About 2,000 people in the United States live with MSUD. It affects males and females equally [8].

MSUD can affect anyone, but people whose parents are closely related are much more likely to have the metabolic disease. For this reason, MSUD frequently occurs among Mennonites in the United States, where members of the community often marry each other. MSUD occurs in 1 of every 380 births in the Mennonite population [9].

MSUD is inherited (passed on) through families. A child is born with MSUD when both parents are carriers of three specific gene mutations (changes) and their child inherits copies of these altered genes – one copy from each parent. These mutations result in little to no activity of enzymes needed to break down three specific amino acids that are in protein-rich foods. These three specific amino acids are leucine, isoleucine and valine. Without the needed enzymes, the three amino acids build up and so do their toxic byproducts (called ketoacids). This leads to the serious health problems seen in MSUD [10].

MSUD occurs more often in communities that have little genetic variation (such as the Mennonite community in the United States). These groups have a higher concentration of people who are carriers of the mutated gene [11].

Symptoms of classic MSUD appear in newborns within 48 hours of birth. In older children, signs of intermediate, intermittent, and thiamine-responsive MSUD

usually develop before age seven. All four types of MSUD have symptoms including: Urine, sweat, or earwax that smells like maple syrup or burnt sugar. (This disorder got its name from this common symptom.) This may not always be present in all types; Poor feeding, vomiting, loss of appetite, irritability; Sluggish/slow/tiredness and weakness; Changes in muscle tone – poor muscle tone, muscle tightness/tension; Abnormal muscle movements, spasms that cause a backward arching of the head, neck and spine; Developmental delay; Seizures, convulsions, respiratory failure and coma (as the condition progresses) [12].

Maple syrup urine disease is a genetic heterogenic disease which relates to deficiency of keto acids dehydrogenase enzyme complex (BCKAD). Four subunits are in the (E1α, E1β, E2 and E3) are in the content of keto acids dehydrogenase enzyme complex (BCKAD). Mutations in three genes coding thoseproteins lead to accumulation of organic keto acids in biological liquids and tissues. Gene, which codes E1a subunit of BCKDHA (branched-chain keto acid dehydrogenase E1, alpha polypeptide), is mapped on the long shoulder of 19 chromosome in position 19q13.1-q13.2; E1b subunit of BCKDHA (branched-chain keto acid dehydrogenase E1, alpha polypeptide) is mapped on the short arm chromosome 6 in position 6q14; E2 DBT (dihydrolipoamide branched-chain trans acylase**)** is mapped on the short arm of chromosome 1 in the position of 1p31; E3 DLD (dihydrolipoamide dehydrogenase) is mapped on the short arm of the chromosome 7 in the position 7q31-q33. Mutation in the E3 DLD (dihydrolipoamide dehydrogenase) gene leads to clinic form which is similar to Lee syndrome [13, 14].

Previous genetic studies have determined that MSUD is an autosomal recessive disease caused by pathogenic variants in genes encoding the E1α, E1β, E2, and E3 components of BCKAD. In 1989, the first genetic variants linked to MSUD were discovered in the E1α subunit (BCKDHA) of the BCKAD complex. Analysis of BCKDH activity in cultured fibroblasts showed that both the father and mother had levels that were 50% of the normal, while the patient's levels were about 5% of normal. DNA sequencing then confirmed that each parent was a carrier for different pathogenic variants in BCKDHA and that the affected index patient was compound heterozygous [15].

Since then, over 190 different pathogenic or likely pathogenic variants have been identified in E1α and the other BCKAD components including E1β (BCKDHB), E2 *(*DBT), and E3 (DLD). All pathogenic variants that have been identified are homozygous or compound heterozygous variants within the same gene [16]. Genetic testing is essential for a clinical diagnosis of MSUD and to de-

termine which subunit is deficient, that may be helpful in the future for determining individualized therapies [17,18].

Frequency of homozygotes in world populations is 1:120000-1:290000, for heterozygotes is 1 for 100-400 newborns. In some isolates frequency of homozygotes is high and comes up to 1:176 newborns. Disease has autosome-recessive type of in heritance. An affected child is born in practically sound parents [19-21]. Thus, the goal of our research is molecular genetic research of two affected kids with the disease of maple syrup urine disease in one Baku family.

Materials and Methods

The venous blood was sampled from 4-year-old girl suffering from maple syrup disease. The patient's urine showed positive reaction to 2, 4-dinitrophenylhydrazine. The child was born on time with normal weight. In the first days of life the urine of a newborn had the scent of maple syrup, and the child had problems of gastro-intestinal tract. The parents of the patient were second degree relatives (the parents were cousins).

Molecular diagnosis was carried out in promoter, exon and intron regions of three genes- BCKDHA (branched-chain keto acid dehydrogenase E1, alpha polypeptide), BCKDHB **(**branched-chain keto acid dehydrogenase E1, beta polypeptide) and DBT (dihydrolipoamide branched-chain trans acylase).

The venous blood for research was taken in a tube containing or heparin. Genomic DNA and RNA kits made by QIAGEN (Germany) company were used for analysis. Intactness and quantity of genomic DNA and PCR reaction products were identified by electrophoresis on 1.7% agarose gel (PowerPacBasicGelDoc^{IM}EZ, USA). PCR reaction was carried out in a following condition: 96^0C- 2 min (96^0C- 30^I, 55^0C- 30^I, 75^0C- 2min). This cycle was repeated 25 times, 72^0C- 10min and 4^0C pause. PCR was carried out in a machine manufactured by the company "Professional Thermocycler Biometra" (Germany). A pair of forward and reverse primers was used for each genomic fragment. For the purification of DNA fragments after the first stage of PCR a set of magnets was used: "AgencourtAMPure XP PCR purification" and SPRIPlate 96 Super Magnet Plate. The second amplification of the purified DNA fragments was carried out in the following condition: 95^0C- 2min, (95^0C-30^I, 55^0C-30^I, 77^0C-2min 25 cycles and 72^0C 10 min, pause 4^0C).

The nucleotide sequence of purified fragments was studied in "GENOMELabGeXP^{TM}Sequencing" (Figure 1). All identified mutations were confirmed using a new PCR product of the abnormal fragment (forward and reverse) (Table 1).

Table 1.The structure of primers used in the diagnosis of MSUD.

Names of primers	Nucleotide primer sequences
1. Sequence- BCKHDA R1	5^{I}-TGA TTC CAT AAA CCTTCC ATA-3^{I}
1. Sequence- BCKHDA F1	5^{I}-TAA CAT CCG ACT GAG ATG GTT ACA-3^{I}
2.Sequence- BCKHDA F2	5^{I}-GGA ATA GAT CGT AAT TGG TAT-3^{I}
2. Sequence- BCKHDA R2	5^{I}-CTA CAG TTA ACA TAG AGG AAT-3^{I}
3. Sequence- BCKHDA F3	5^{I}-CAT AAT CCA TTC AAC TGT TAA-3^{I}
3. Sequence- BCKHDA R3	5^{I}-ACA TAG TCG TGT CGA GTC CAG TAA-3^{I}
4. Sequence- BCKHDA F4	5^{I}-TTC TGG TAA GTA CTT AGA GGA-3^{I}
4. Sequence- BCKHDA R4	5^{I}-GGA TAG ACA AGA GAT GCT GGA-3^{I}
5. Sequence- BCKHDB F1	5^{I}-GGG TCA AAT GTA TAG GGC CAC-3^{I}
5. Sequence- BCKHDB R1	5^{I}-TCG TTT GCG AGT ATA GCA TAT-3^{I}
6. Sequence- BCKHDB F2	5^{I}-ACT GCA CTT CTC TTC ATC CAC CTG-3^{I}
6. Sequence- BCKHDB- R2	5^{I}-TCA AGG TTG GCG ATG ATC TAAGT-3^{I}
7. Sequence- BCKHDB- F3	5^{I}-AGA TAG TCA TGA GAA GCT GGT-3^{I}
7. Sequence- BCKHDB- R3	5^{I}-TTA ACA GAT CTT GAT TGG TAG-3^{I}
8. Sequence- BCKHDB- F4	5^{I}-CCA ATT TCG AGT ATC GCG TAA-3^{I}
9. Sequence- BCKHDB- R4	5^{I}-CCT GCG CTA CTT GTC GTC CAC CTA-3^{I}

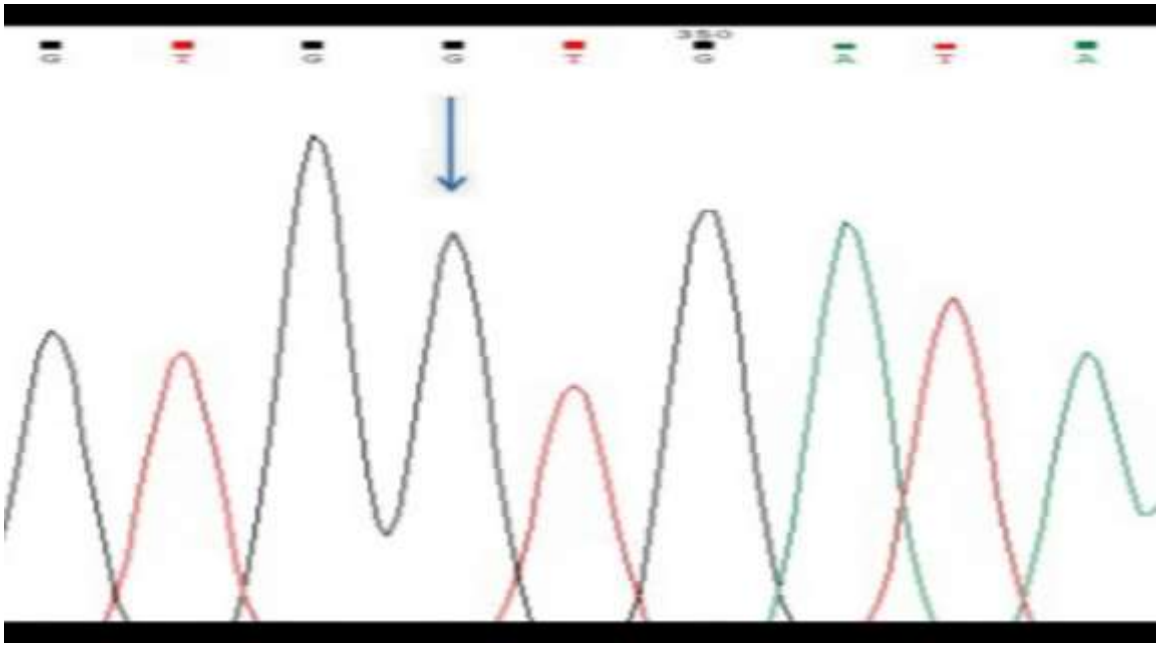

Figure 1. Electropherogram of changes in the nucleotide sequence of the homozygous form of the new mutation 1221 (A> G), found in the 10th exon of the DBT gene.

Research Results and Their Discussion

The amount of Valine, Leucine and Isoleucine amino acids in the urine was less than normal: Valine result- 498.66mkmol/gKre (normal range 9900-31600 mkmol/gKre), Isoleucine result- 395.97 mkmol/gKre (normal range 3800 - 31200 mkmol/gKre) and Leucine result- 2032.98 mkmol/gKre (normal range 7000 - 57000 mkmol/gKre). Others amino acids in the urine was different: Ornithine result-26.15 mkmol/gKre (normal range 55.00-164.00 mkmol/gKre). Cystine result-71.13 mkmol/gKre (normal range 68.00-710.00 mkmol/gKre). Tyrosine result-70.76 mkmol/gKre (normal range 333.00- 1550.00 mkmol/gKre). Methionine result-20.66 mkmol/gKre (normal range 174.00 1690.00 mkmol/gKre). Allo-isoleucine result-153.04 mkmol/gKre (normal range 0.00-29.00 mkmol/gKre). Phenylalanine result-64.35 mkmol/gKre (normal range 175.60-1340.00 mkmol/gKre). Tryptophan result-40.51 mkmol/gKre (normal range 0.00-93.00 mkmol/gKre). The amount of Valine, Isoleucine and Leucine amino

acids in the patient's urine was 1.6; 1.3 and 3.6 times higher than the highest value of normal range, respectively.

The amount of valine, leucine and isoleucine amino acids in the plasma was much higher than the normal range: valine result- 808.55mkmol/l (normal range 64.00-296.00 mkmol/l), isoleucine result- 636.13 mkmol/L (normal range 31.00-81.20 mkmol/L) and leucine result- 3782.02 mkmol/L (normal range 47.00-150.00 mkmol/L). Other amino asids in the plasma was normally: Cystine result-26.33mkmol/L (normal range 16.00-87.00 mkmol/L), Lysine result- 58.09 mkmol/L (normal range 52.00-90.00 mkmol/L). Tyrosine result-49.54 mkmol/L (normal range 22.00-105.00 mkmol/L). Methionine result-10.67 mkmol/L (normal range 40.00 mkmol/L). Allo-isoleucine result-276.10 mkmol/L (normal range 0.00-290.00 mkmol/L). Phenylalanine result-56.26mkmol/L (normal range 31.00-75.00mkmol/L). Tryptophan Tryptophan result-16.29mkmol/L (normal range 23.00-71.00 mkmol/L).

The amount of valine, isoleucine and leucine in the patient's plasma was 2.7; 7.9 and 25.2 times higher than the highest value of normal range, respectively.

Thus, for the diagnosis of maple syrup metabolic disorder the amount of Valine, Leucine and Isoleucine amino acids was evaluated in the urine and plasma. The comparison of obtained results revealed that plasma analysis was more informative.

Molecular analysis of three genes-BCKDHA (branched-chain keto acid dehydrogenase E1, alpha polypeptide), BCKDHB **(**branched-chain keto acid dehydrogenase E1, beta polypeptide) and DBT (dihydrolipoamide branched-chain trans acylase) genes- causing maple syrup metabolic disorder was carried out. According to literatures, out of 50 mutations, 45% was found in BCKDHA (branched-chain keto acid dehydrogenase E1, alpha polypeptide) gene (MSUD type 1A), 35% of mutations in BCKDHB **(**branched-chain keto acid dehydrogenase E1, beta polypeptide) gene (MSUD type 1B) and 20% of that in DBT (dihydrolipoamide branched-chain trans acylase**)** gene (MSUD type 2).

No mutations were found in BCKDHA (branched-chain keto acid dehydrogenase E1, alpha polypeptide) and BCKDHB **(**branched-chain keto acid dehydrogenase E1, beta polypeptide) genes. Substitution of Adenine by Guanine mutation has been found in DBT (dihydrolipoamide branched-chain trans acylase**)** gene at 1199 nucleotide position (p. N400S 1199 A (Adenine)-G(Guanine)). This mutation was homozygous.

Thus, identified mutation (1199 A (Adenine) - G (Guanine)) is a new mutation that is not shown in any literature. This homozygous mutation causes maple syrup metabolic disorder by disrupting the metabolism of Valine, Leucine and Isoleucine.

The identification of maple syrup metabolic disorder by biochemical and molecular genetic methods in Azerbaijan population confirms the importance of screening test of newborns for the said disease.

Conflict of Interest: The authors declare that there is no conflict of interest that could be perceived as prejudicial to the impartiality of the reported research.

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