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Molecular Genetic Diagnosis of Inherited Metabolic Disease Named Maple Syrup Scent

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Abstract

Homozygous substitution of adenine by guanine mutation has been found in DBT gene at 1199 nucleotide position (p. N400S 1199 A-G). No mutations were identified in BCKDH and BCKDHB genes. The identified mutation (1199 A-G) 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. The presence of maple syrup disease in Azerbaijan population confirms the importance of screening test of newborns for respective disease.

Keywords: ***Inherited metabolic disorder; Maple syrup metabolic disorder; Mutation; Neutral genetic polymorphisms***

Introduction

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.

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 (E1a, E1b, E2 and E3) are in the content of BCKAD. Mutations in three genes coding those proteins lead to accumulation of organic keto acids in biological liquids and tissues. Gene, which codes E1a subunit of BCKDHA, is mapped on the long shoulder of 19 chromosome in 19q13.1-q13.2 position; E1b subunit of

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BCKDHA is mapped on the short arm chromosome 6 in position of 6q14; E2 DBT is mapped on the short arm of chromosome 1 in the position of 1p31; E3 DLD is mapped on the short arm of the chromosome 7 short shoulder in the position of 7q31-q33. Mutation in the E3 DLD gene leads to clinic form which is similar to Lee syndrome [1-6].

50 mutations of those genes are known. 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 [3-5].
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 taken from 4-yr-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, BCKDHB and DBT.
The venous blood for research was taken in a tube containing EDTA 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 (Power Pac Basic Gel Doc I MEZ, USA) [5,6].

PCR reaction was carried out in a following condition: 96°C-2 min (96°C-30^{I}, 55°C-30^{I}, 75°C-2min. This cycle was repeated 25 times), 72°C-10min and 4°C pause. PCR was carried out in a machine produced by the company of "Professional Thermocycler Biometra" (Germany). A pair of forward and reverse primers were used for each genomic fragment. The purification of DNA fragments after the first stage of PCR a set of magnets was used: "Agencourt AM Pure XP PCR purification" and SPRI Plate 96 Super Magnet Plate. The second amplification of the purified DNA fragments was carried out in the following condition: 95°C-2min, (95°C-30^{I}, 55°C-30^{I}, 77°C-2min 25 cycles and 72°C 10 min, pause 4°C. The nucleotide sequence of purified fragments was studied in "GENOMEL ab Ge XPTM Sequencing".

Results and Discussion

The amount of amino acids in the urine and blood plasma of the patient T.E. are shown in the TABLES 1 and 2.

As can be seen from the TABLE 1, except cystine and tryptophan, the amount of other amino acids was different from the normal amount. The amount of valine, leucine and isoleucine amino acids in the urine was less than normal: valine result-

TABLE 1. **The amount of amino acids in the urine of patient T.E.**

The name of amino acid	Amount	
	Result	**Norm**
Ornithine	26, 15 mkmol/gKre	55, 00-164,00 mkmol/gKre
Cystine	71, 13 mkmol/gKre	68, 00-710,00 mkmol/gKre
Lysine	24, 90 mkmol/gKre	189, 00-850,00 mkmol/gKre
Tyrosine	70, 76 mkmol/gKre	333, 00-1550,00 mkmol/gKre
Methionine	20, 66 mkmol/gKre	174, 00-1690,00 mkmol/gKre
Valine	498, 66 mkmol/gKre	99, 00-316,00 mkmol/gKre
Isoleucine	395, 97 mkmol/gKre	38, 00-312,00 mkmol/gKre
Allo-isoleucine	153, 04 mkmol/gKre	0, 00-29, 00 mkmol/gKre
Leucine	2032, 98 mkmol/gKre	70, 00-570, 00 mkmol/gKre
Phenylalanine	64, 35 mkmol/gKre	175, 60-1340, 00 mkmol/gKre
Tryptophan	40, 51 mkmol/gKre	0, 00-93, 00 mkmol/gKre

498, 66 mkmol/gKre (normal range, 9900 mkmol/gKre-31600 mkmol/gKre), isoleucine result-395, 97 mkmol/gKre (normal range, 3800 mkmol/gKre-31200 mkmol/gKre) and leucine result-2032, 98 mkmol/gKre (normal range, 7000 mkmol/gKre-57000 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.

TABLE 2. **The amount of amino acids in the plasma of patient T.E.**

The name of amino acid	Amount	
	Result	**Norm**
Cystine	26, 33 mkmol/L	16, 00-87, 00 mkmol/L
Lysine	58, 09 mkmol/L	52, 00-90, 00 mkmol/L
Tyrosine	49, 54 mkmol/L	22, 00-105, 00 mkmol/L
Methionine	10, 67 mkmol/L	40, 00 mkmol/L
Valine	808, 55 mkmol/L	164, 00-296, 00 mkmol/L
Isoleucine	636, 13 mkmol/L	31, 00-81, 20 mkmol/L
Allo-isoleucine	276, 10 mkmol/L	0, 00-290, 00 mkmol/L
Leucine	3782, 02 mkmol/L	47, 00-150, 00 mkmol/L

Phenylalanine	56, 26 mkmol/L	31, 00-75, 00 mkmol/L
Tryptophan	16, 29 mkmol/L	23, 00-71, 00 mkmol/L
Phenylalanine/tyrosine	1, 14	00, 00

The amount of valine, leucine and isoleucine amino acids in the plasma was much higher than the normal range: valine result-808, 55 mkmol/l (normal range 64, 00 mkmol/l-296, 00 mkmol/l), isoleucine result-636, 13 mkmol/L (normal range 31, 00 mkmol/l-81, 20 mkmol/L) and leucine result-3782, 02 mkmol/L (normal range 47, 00 mkmol/l-150,00 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, BCKDHB and DBT genes-causing maple syrup metabolic disorder was carried out. According to literatures, out of 50 mutations, 45% was found in BCKDHA gene (MSUD type 1A), 35% of mutations in BCKDHB gene (MSUD type 1B) and 20% of that in DBH gene (MSUD type 2) [7].

No mutations were found in BCKDHA and BCKDHB genes. Substitution of adenine by guanine mutation has been found in DBT gene at 1199 nucleotide position (p. N400S 1199 A-G). This mutation was homozygous.

Thus, identified mutation (1199 A-G) 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 respective disease.

Conclusions

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.

Substitution of adenine by guanine mutation has been found in DBT gene at 1199 nucleotide position (p. N400S 1199 A-G). This mutation was homozygous. No mutations were found in BCKDHA and BCKDHB genes.

The identified mutation (1199A-G) is a new mutation that has not been described in any literature. In homozygous state it disrupts the metabolism of valine, leucine and isoleucine amino acids and causes maple syrup metabolic disorder.

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 respective disease.

REFERENCES

1. Geneva. Report to the scientific group of the WHO. Fight against hereditary diseases. WHO Report. 1997;865:133.
2. Application information: Purification of GENOMELABTM GeXP Sequencing Productions using SPRIClean SEQ^{R} Magnetic Beards. CEQ 2000, CEQ 2000XL, CEQ 8000, CEQ 8800 & GeXP Instruments BECKMAN COULTER. Application Team Europe.
3. Chi CS, Tsai CR, Chen LH, et al. 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. Europ J Hum Genet. 2003;11:931-36.
4. Chuang JL, Wynn RM, Moss, et al. Structural and biochemical basis for novel mutations in homozygous Israeli maple syrup urine disease patients. J Biol Chem. 2004;279:17792-800.
5. Mersey BD, Jin P, Danner DJ. Human microRNA (miR29b) expression controls the amount of branched chain alpha-ketoacid dehydrogenase complex in a cell. Hum Molec Genet. 2005;14:3371-77.
6. Mc Kusick A. Mendelian inheritance in man. 10th ed, London. 2002;2115.
7. Huseynova LS, Rasulov EM, Aliyeva KA. A new case of Bckdhb 508 (CT) homozygous gene mutation in maple syrup urine disease. Advances in Biology & Earth Sciences. 2017;2: 248-54.