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Original Article

Establishing genetic spectrum of *MSUD* by screening of *BCKDHA*, *BCKDHB*, *DLD and DBT* in different ethnic groups of Azerbaijan population

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Abstract

Mutations in *BCKDHA*, *BCKDHB*, *DLD* and *DBT* are associated with Maple Syrup Urine Disease (MSUD) in different populations worldwide, therefore screening of these genes is crucial for genetic counseling, diagnosis and treatments of MSUD in patients suffering from MSUD. The present study was therefore carried out by screening MSUD patients from different regions including Baku, Sheki-Zagatala, Guba-Khachmaz, Lankaran-Astara zones to establish the genetic spectrum of MSUD patients of in Azerbaijan population. The cohort consisted of 800 patients clinically diagnosed with MSUD from the year 2015 to 2020. The gene panel sequencing (*BCKDHA*, *BCKDHB*, *DLD* and *DBT*) was performed by amplification of exonic sequences using the polymerase chain reaction (PCR) method followed by Sanger sequencing of the amplified product. For the confirmation of identified mutation a control panel of 140 healthy individuals was also collected from the population. The sequencing of the exonic sequence of the four genes resulted in identification of four missense variants. One patient carried compound heterozygous variants 1221(A>G) and 972 (C>T) in exon 9 and 10 respectively while another patient had homozygous 508(C>T) variant in *BCKDHB*, and homozygous 1199(A>G) was found in *DBT* in another patient. All the three patients carrying the identified mutations were from Guba-Khachmaz zone: one was Azerbaijani Turk, and two were Lezgi ethnic group. The *in silico* analysis predicted the variants to be pathogenic for protein function. In conclusion, the present study highlights the genetic role of *BCKDHB* and *DBT* in onset of MSUD in Azerbaijan especially Guba-Khachmaz zone where *BCKDHB* variants found to be more common in MSUD patient with followed by *DBT*. The identified variants were absent from the control group, thus suggesting their role in disease manifestation. Out of the 800 MSUD screened patients excluded cases might be having deep intronic mutation either in *BCKDHB* and *DBT* or other gene or might be having novel gene which could only be identified through exome/genome sequencing of the remaining patient.

Keywords: MSUD, Genetic spectrum, Azerbaijani Turk, Guba-Khachmaz zone, Rare mutations, Ethnic specificity

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Introduction

Maple Syrup Urine Disease (MSUD) is a metabolic disorder caused by a deficiency of a specific enzyme within cells. In normal human metabolism, 22 amino acids—essential for body functions and the development of organs and muscles—are derived from dietary proteins. When proteins are consumed, they are digested and broken down into these amino acids. In individuals with MSUD, a defect in a particular enzyme prevents the proper breakdown of the branched-chain amino acids (leucine, isoleucine, and valine). Normally, an enzyme complex in cells cleaves these amino acids into their individual chemical components. However, in MSUD, this enzyme is nonfunctional, leading to a buildup of these amino acids and their toxic byproducts, which can damage tissues—especially the brain—and impair bodily functions (Mersey et al., 2005; Carecchio et al., 2011). Symptoms of MSUD typically manifest immediately after birth. Physical examination may reveal dystonia, hepatomegaly, and motor-postural delays. Early molecular genetic diagnosis and medical intervention are vital for managing the disease effectively. These insights contribute significantly to understanding MSUD, enabling many affected families and children to benefit from early diagnosis. Molecular genetic testing is essential for confirming the diagnosis and guiding treatment, as well as providing valuable information for genetic counseling of affected families.

MSUD generally inherited in an autosomal recessive disorder (Quental et al., 2008; Quental et al., 2009). Caused by mutations in the genes namely BCKDHA (19q13.1–13.2), BCKDHB (6q14), DLD (7q31–q33), and DBT (1p31) genes. Mutations in these genes disrupt the function of enzymes involved in the metabolism of leucine, isoleucine, and valine. This disruption causes an accumulation of these amino acids and their toxic derivatives in the body, which can adversely affect organs and tissues (Puffenberger, 2003; Morton et al., 2002; Ogier de Baulny and Saudubray, 2002; Zinnanti et al., 2009; Wessel et al., 2015; Phan et al., 2006; Tsai et al., 2014; Huseynova et al., 2019). Since the discovery of these mutations, over 190 different variants have been identified. Early detection and prompt treatment are crucial; if left untreated, the disturbance in amino acid metabolism can worsen the patient's condition and may lead to death (Hallam et al., 2005; Mazariegos et al., 2012; Muelly et al., 2013).

While MSUD is considered an ethnic disorder with a worldwide prevalence of approximately 1 in 185,000 live births, the incidence is notably higher among the Mennonite population in Pennsylvania, about 1 in 176 births (Carecchio et al., 2011). In view of ethnic specific manifestation of MSUD, we screened 800 MSUD patient from different ethnic groups of Azerbaijan population. This study identified ethnic-specific mutation patterns in the BCKDHB and DBT genes within Azerbaijan population.

Material and Methods

Ethical consent and sample collection

All participants provided written informed consent affirming their voluntary participation in the study. This commitment ensured ethical transparency and protected patient rights. The study underwent review and approval by the Ethics Committee of Azerbaijan Medical University (approval number *AMU/EC/16/04/2021/N16*), providing additional ethical assurance for this research undertaking and it was performed according to the Declaration of Helsinki. A total of 940 participants (800 MSUD patients and 140 control samples) were recruited in the present study with random collection from from different zones of Azerbaijan. The biochemical profiling from the blood and urine of the patients confirmed the amino acid defect typical of MSUD.

Genetic analysis

For the genetic analysis, A total of 2 cc of blood was taken from the participating individuals and DNA was isolated through kit method as per manufacturer protocol ([200 mkl of QIA ampgenomic DNA and RNA kit (QIAGEN. Germany), buffer solution, 200 mkl of venous blood and 20 mkl of protease enzyme (QIAGEN)]. For the primer designing the exonic sequences along with the flanking intronic sequences of the selected genes using the Primer3 algorithm (http://frodo.wi.mit.edu/primer3) according to the following GenBank cDNA and genomic DNA accession numbers: NM_000709.3 and NG_013004.1 for the BCKDHA gene, NM_000056.3 and NG_009775.1 for the BCKDHB gene, and NM_001918.3 and NG_011852.2 for the DBT gene. All primer sequences are presented in supplementary table 1 while and PCR reaction conditions are available on request. PCR was performed in the "T100TM Thermal BIO RAD" (Germany) amplifier.

A pair of Forward (F) and Reverse (R) primers were used for each genome fragment (Huseynova et.al., 2021). The amplified products were electrophoresed on 1.5% agarose gel. For this purpose, Power PacBasic Gel DocIM EZ (BioRad. USA) electrophoresis and Lambda DNA Mixed Digest marker were used. The PCR purified products were Sanger sequenced using HiSeq2500 sequencer. Variations were screened with indicators Sift_Pred=Damaging. Sequence of DNA fragments was studied on "Applied Biosystems (Hitachi) 3130xl Genetic Analyzer Sequencing" sequencer. The data obtained in Sanger sequencing were compared with the reference gene using the "SeqScape TM" program.

In silico prediction of identified variants

For the *in silico* prediction to study the effect of mutation on protein function and its role in manifestation of MSUD, following in silico steps were performed (www.swiss-prot.org): gene sequences were obtained from NCBI databases. Specific mutations were identified using ClinVar and HGMD databases. Experimental structures from the Protein Data Bank (PDB) were used to model the native protein structures. Mutations were introduced into the 3D structures using PyMOL software. The effects of these mutations were predicted with PolyPhen-2. To assess how mutations influence protein stability, FoldX was employed. The interactions of mutant proteins with cofactors or substrates were simulated using AutoDock. Molecular dynamics simulations of both wild-type and mutant proteins were carried out with GROMACS, analyzing stability, flexibility, and conformational changes. Mutations and their structural effects were visualized with PyMOL, and mutation impacts were further evaluated using the Missense3D database for automated structural impact predictions. Additionally, KBase was utilized to study how mutations in the BCKDH complex genes affect protein structure.

Results

All the patients screened in the present study (Table 1), presented hallmark symptoms of MSUD including distinctive maple syrup odor in the urine, earwax, sweat, and along with poor feeding, lethargy and irritability. Skin lesions due to nutritional deficiencies or malabsorption were prominent. Considering the symptoms, the severity of the disease was compared according to mild and severe form of the disease. The MSUD symproms were observed only in severe and mild forms. There is no significant difference between the number of severe and mild forms of MSUD patients ($p>0.05$). A moderately severe form was not observed among MSUD patients.

Table-1: Studied cohort overview and muation identification.

Studied Cohort	**Number**	**Identified Mutations in Studied Panel**					
		BCKDHA	***BCKDHB***		***DBT***		***DLD***
MSUD Patients	800	0	3	Exon 2: ***(508(C>T)*** Homozygous Exon 9/10: ***972(C>T)/1221(A>G)*** Compound Heterozygous	1	Exon 10: ***(1199(A>G)*** Homozygous	0
Healthy Controls	140	0	0		0		0
Total	940	0	3		1		0

Out of 800 MSUD patients, the genetic screening resulted in identification of mutation in three patients from different families (Table 1) from the Guba-Khachmaz zone. The amino acid metabolic dysfunction specifically leucine metabolic defect associated mutations were found in 3 patients. In the first patient (Figure 1A), the 508(C>T) mutation affected the protein helix. A homozygous C>T

substitution at position 508 in exon 2 of the *BCKDHB* gene caused a (Lys-Gly) lysine-glutamine change at position 332 of the corresponding polypeptide. In the second patient (Figure 1B), a compound heterozygous mutation was detected that resulted in C>T substitution at position 972 of exon 9 of the *BCKDHB*, and an arginine-serine change at position 673 of the polypeptide and a substitution A>G at position 1221 of exon 10, phenylalanine-glutamine at position 947 of the polypeptide (Figure 1B). Another mutation was detected in patient 3 (Figure in a 4-year-old girl from Khachmaz region of Azerbaijan, observed to carry a homozygous change 1199(A>G) in exon 10 of *DBT* resulting from replacement of adenine by guanine at position 1199 causing a lysine-glutamine to change at position 907 of the protein.

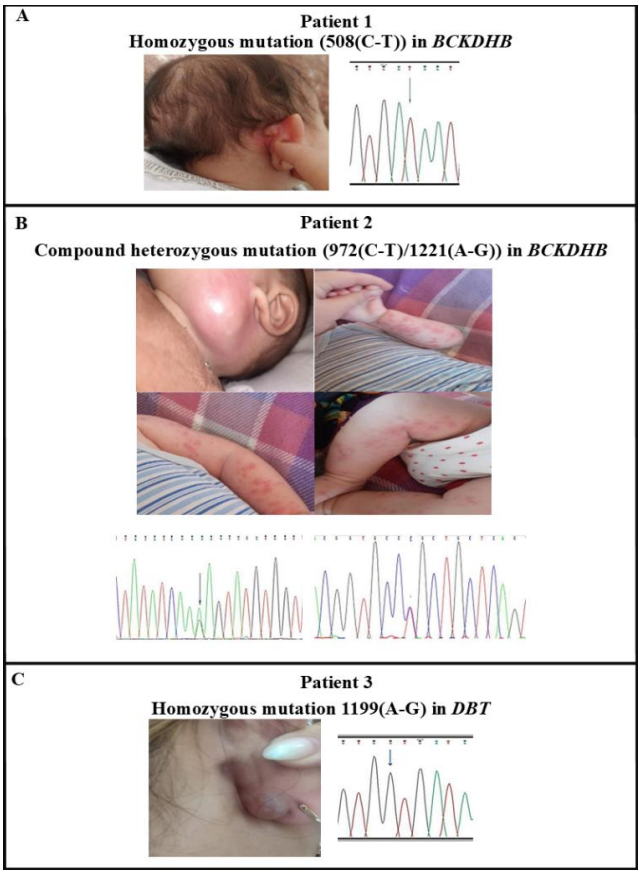

Figure-1. Clinical presentation and identified mutations in three patients from the MSUD studied panel. A: Patient 1 with (508(C>T)) homozygous mutation of the *BCKDHB*. B: Patient 2 with a compound heterozygous *BCKDHB* variants (972(C>T)/1221(A>G)). C: Patient 3 with homozygous 1199 (A>G) mutation in *DBT*.

All these four mutations that were identified in three patients were newborns where the disease manifested with characteristic symptoms from the day of birth. As the biochemical diagnostic tests displayed the presence of valine, isoleucine and leucine amino acids in the blood and urine of these newborns prior to genetic testing, the genetic analysis therefore confirmed the mutations to be associated with the metabolic amino acid dysfunction these three patients (Figure 1, Table 1). The identified mutations were absent in the 140 controls.

The geographical localization of 2 out of 3 patients is from Khinalig village of Guba, and one from Khachmaz district. Ethnic specificity was therefore observed in the present study with the frequency and spectrum of studied mutations in *BCKDHB* and *DBT* genes on the territory of Azerbaijan. One of these patients is an Azerbaijani Turk, and two are from the Lezgi ethnic group. Thus, the frequency of MSUD disease is 0.001 among Azerbaijani Turks and 0,0021 in the Lezgi ethnic group respectively. The frequency of mutations of these genes studied in the population of Azerbaijan was equal to 0.001.

Discussion

The present study aimed to explore the mutational spectrum of *BCKDHA*, *BCKDHB*, *DLD*, and *DBT* genes in Azerbaijani patients diagnosed with MSUD. Using polymerase chain reaction (PCR) and exome sequencing analysis, we identified novel mutations in three patients. Specifically, we analyzed exons 2, 9, and 10 of the *BCKDHB* gene and exon 10 of the *DBT* gene.

Previous studies have highlighted the genetic heterogeneity of MSUD across different populations. For instance, an analysis of 52 MSUD patients from Saudi Arabia revealed 20 novel mutations in *BCKDHA*, distributed across the entire coding region, all predicted to be pathogenic. Notably, no *DLD* mutations were detected in this cohort (Faiqa et al., 2017). Similarly, research on Vietnamese families with MSUD uncovered novel pathogenic variants in *BCKDHA*, contributing to the understanding of MSUD's genetic diversity (Thi et al., 2020). In a Brazilian cohort, novel variants in *BCKDHB* (p.Gly131Val, p.Glu146Glnfs13, p.Phe149Cysfs9, p.Cys207Phe, p.Lys211Asn) and *DBT* (p.Glu148Ter, p.Glu417Val) were identified, expanding the known mutational spectrum (Ana et al., 2020). Additionally, a Chinese case study reported a novel *BCKDHB*

mutation (c.391G>A, p.Gly131Arg) alongside a large gene deletion, providing further insights into MSUD's genetic complexity (Gang et al., 2018).

In our study, we detected the *1199A>G* mutation in the *DBT* gene in homozygous form in one patient. A second patient carried a homozygous *508C>T* mutation in *BCKDHB*, while a third exhibited compound heterozygous mutations (*972C>T* in exon 9 and *1221A>G* in exon 10) in the same gene. Structural analysis revealed that the *508C>T* mutation in *BCKDHB* slightly alters the protein helix, introducing an additional small helix. This substitution results in a lysine-to-glutamine change at position 332 (p.Lys332Gln). The two missense mutations (*p.Arg673Ser*, *p.Phe947Gly*) were located in coil regions, while *p.Lys907Gly* extended a β-sheet. Additionally, the *1199A>G* mutation in *DBT* led to a lysine-to-glutamine substitution at position 508 (p.Lys508Gln) (Huseynova et al., 2017; Huseynova et al., 2019).

To our knowledge, this is the first comprehensive study of *BCKDHA*, *BCKDHB*, *DLD*, and *DBT* mutations in the Azerbaijani population. We identified four rare mutations in *BCKDHB* and *DBT* among three MSUD patients, none of which had been previously reported from Azerbaijani population.

Azerbaijan is home to diverse ethnic groups, primarily residing in the Sheki-Zagatala, Guba-Khachmaz, Lankaran-Astara regions, and Baku. Between 2015 and 2020, 940 individuals from these areas were included in our study. Interestingly, all three patients with novel mutations were from the Guba-Khachmaz zone—one of Azerbaijani Turkic origin and two from the Lezgi ethnic group. Our findings suggest a higher prevalence of MSUD among the Lezgi population (0.0021), indicating that *BCKDHB* and *DBT* may be common causative genes for MSUD in Azerbaijan. Consequently, we recommend prioritizing these genes in future molecular screenings, particularly for the Lezgi ethnic group (Feuchtbaum et al., 2012; Huseynova, 2017).

The identification of these mutations holds significant implications for molecular diagnosis, enabling preventive strategies such as carrier testing, prenatal screening, premarital genetic counseling, and preimplantation genetic diagnosis in Azerbaijan (Huseynova et al., 2017; Huseynova et al., 2019).

Conclusion

In this study we report the molecular genetic analysis of MSUD patients from Azerbaijan. The analysis resulted in identification of rare variants two of which were present compound heterozygously in one patient. while the two mutations were homozygous. All the mutation were inherited recessively from healthy parent thus highlighting the role of high degree of inbreeding in the Azerbaijan population. The patients displayed clinical heterogeneity that was from mild and severe forms of the disease. The 3 patients (1 male and 2 female) with detected mutations were from Guba-Khachmaz region thus showing ethnic specificity.

To date, genetic study of MSUD has not been conducted in the population of Azerbaijan. As MSUD is among the clinical sub-class of inborn error of metabolism, and present study being the first report of molecular genetic analysis of MSUD, therefore, the present study highlights the importance of genetic based prenatal diagnosis and new-born screening program for early detection and management of MSUD in the Azerbaijan population.

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Conflict of Interest: None.
Source of Funding: None.

Ethical Approval Statement and Consent to Participate

Informed consent was acquired from the participants. The study underwent review and approval by the Ethics Committee of Azerbaijan Medical University (approval number AMU/EC/16/04/2021/N16), providing additional ethical assurance for this research undertaking.

Contribution of Authors

Huseynova LS: Study director, conducted experiments, collected and analyzed data and wrote the manuscript.

Suleymanova LM: Performed statistical analysis and wrote the manuscript.

Mammadova SN & Valiyeva GA: Reviewed literature, conducted experiments and performed analyses.

All authors have read and approved the final draft of the manuscript.

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Supplementary Material

Table-S1. Primers used for the BCKDHA gene.

Ekzon	**Primers name**	**Primers**	t ºC	Amplicon size (b.p)
1	BCKDHA_EX1_F	5'-CTGGTCAGGTTGCCCTCTT-3'	60ºC	421
	BCKDHA_EX1_R	5'-GGACCCCACACTCTGAAGATAG-3'		
2-3	BCKDHA_EX2_3F	5'-CACATGCTCAACCACCATG-3'	62ºC	561
	BCKDHA_EX2_3R	5'-CTTGGGAGCCATTCCTTTG-3'		
4	BCKDHA_EX4_F	5'-ACAGCAACTCGATCCCTCTG-3'	60ºC	394
	BCKDHA_EX4_R	5'-CTGCTCCTGGAAGAACACTCA-3'		
5	BCKDHA_EX5_F	5'-CTTTCCTGTCTGCCTGCC-3'	62ºC	392
	BCKDHA_EX5_R	5'-AGCACAGACCAGGGCTCTAG-3'		
6	BCKDHA_EX6_F	5'-CATCAGGAGCTGAGGTGTTTC-3'	60ºC	460
	BCKDHA_EX6_R	5'-ACAGGACGAGAACCAGGAAG-3'		
7	BCKDHA_EX7_F	5'-GGAGTTGAGGTCCTGAGCAC-3'	60ºC	406
	BCKDHA_EX7_R	5'-AGGAGGAGTGGAAACGGAAT-3'		
8	BCKDHA_EX8_F	5'-ACTGACAGCCACCGTAGCAT-3'	62ºC	441
	BCKDHA_EX8_R	5'-GGTGTTCCACAAATCCTTCC-3'		
9	BCKDHA_EX9_F	5'-GAGTGGTTAATTCCTTGCCAAG-3'	60ºC	684
	BCKDHA_EX9_R	5'-ACTCCAGGAAACAAAGACCAG-3'		

Table-S2. Primers used for the BCKDHB gene.

Ekzon	**Primers name**	**Primers**	t ºC	Amplicon size (b.p)
1	BCKDHB_EX1_F	5'-CTAGCCCACACTTCCCCTCT-3'	63ºC	552
	BCKDHB_EX1_R	5'-GCAAGGAGGTTCCAGAGAGTC-3'		
2	BCKDHB_EX2_F	5'-CTCCAGGTCTGTATTGCTTTTGT-3'	60ºC	383
	BCKDHB_EX2_R	5'-GCCCCAATCATACCTTTGAA-3'		
3	BCKDHB_EX3_F	5'-GTGTGTGTGGTAACTGTCATCCA-3'	60ºC	684
	BCKDHB_EX3_R	5'-CCCAACAGGCAGAATCTCC-3'		
4	BCKDHB_EX4_F	5'-CCTGTTCTATACTTCTCCATCCC-3'	60ºC	307
	BCKDHB_EX4_R	5'-GGGTAGCGGCAATACTTGAA-3'		
5	BCKDHB_EX5_F	5'-AGGAAGAACGGAAGGAGATTG-3'	60ºC	412
	BCKDHB_EX5_R	5'-AACTGGGCATTGGATAGCATA-3'		
6	BCKDHB_EX6_F	5'-GCCCTTCTTAGCAGCGAGT-3'	60ºC	405
	BCKDHB_EX6_R	5'-CAGATTTCCTCTTTGTTTCCACA-3'		
7	BCKDHB_EX7_F	5'-GCACAAGTGTCACCTCAGAAAA-3'	60ºC	482
	BCKDHB_EX7_R	5'-ATAGATCTGAAGTGTCCTCGCC-3'		
8	BCKDHB_EX8_F	5'-CTCCATGCAGATCAGTTCCTG-3'	60ºC	484
	BCKDHB_EX8_R	5'-GCATAAAGGACCCCATTTTGTA-3'		
9	BCKDHB_EX9_F	5'-CCTGTCGAAAGCGAGTTGTAAC-3'	60ºC	311
	BCKDHB_EX9_R	5'-CTTCTGGAATTGGCATGTGG-3'		
10	BCKDHB_EX10_F	5'-CGAACATGCTGTTACCTGCTT-3'	60ºC	391
	BCKDHB_EX10_R	5'-CTGATGATTGCTGTGTCTTGG-3'		
11	BCKDHB_EX11_F	5'-AGCCAAGGTAGTGATGGTGG-3'	63ºC	690
	BCKDHB_EX11_R	5'-CATCCTGGTCATAAAGAACTGAAC-3'		

Table-S3. Primers used for the DLD gene.

Ekzon	**Primers name**	**Primers**	t ºC	Amplicon size (b.p)
1	DLD_EX1_F	5'-CTTCCCTCCCTATTGGTCG-3'	60ºC	316
	DLD_EX1_R	5'-CTCCGTTCTCTGCCCTTTATT-3'		
2	DLD_EX2_F	5'-CAGAAGGAATTTTGGGTAAGGA-3'	62ºC	563
	DLD_EX2_R	5'-CAATCTGGGCAACTGAGTGA-3'		
3	DLD_EX3_F	5'-GCCTCTGCCTGAGAACATTC-3'	60ºC	376
	DLD_EX3_R	5'-TGTATGACAAAGTCCTTCACCACT-3'		
4	DLD_EX4_F	5'-CTGAAAGTAAATGCTGGGCTAGA-3'	60ºC	552
	DLD_EX4_R	5'-ATCTCTTCCTTTTGCTATTGCCT-3'		
5	DLD_EX5_F	5'-CCCACTCTACCCATACCATTAGG-3'	60ºC	606
	DLD_EX5_R	5'-AGCACCTGACATAAGACCTGGT-3'		
6	DLD_EX6_F	5'-CCTGATGGTTACCACATGCA-3'	60ºC	483
	DLD_EX6_R	5'-TCTACTGAGGTAGCTTCCCCC-3'		
7	DLD_EX7_F	5'-GCAGTCAGTGTTCCAGCTTTG-3'	60ºC	405
	DLD_EX7_R	5'-CAAATAAATGTCCTACTCAAGCCTT-3'		
8	DLD_EX8_F	5'-GGAACTTTGGCTGGTCTGTATC-3'	60ºC	660
	DLD_EX8_R	5'-GCTGCTTCTTTTTGAGAGGGT-3'		
9-10	DLD_EX9_10_F	5'-ATGGCAGTGAAGGTTGATCC-3'	60ºC	542
	DLD_EX9_10_R	5'-TGTGTTTAGTCCCTGAATTTGCT-3'		
11	DLD_EX11_F	5'-GGTTTGCCTGATCTTACACCA-3'	60ºC	469
	DLD_EX11_R	5'-CCCAGGAGAACCATTACACC-3'		
12	DLD_EX12_F	5'-CTGAAAGTAAATGCTGGGCTAGA-3'	60ºC	307
	DLD_EX12_R	5'-ATCTCTTCCTTTTGCTATTGCCT-3'		
13	DLD_EX13_F	5'-CCCACTCTACCCATACCATTAGG-3'	60ºC	412
	DLD_EX13_R	5'-AGCACCTGACATAAGACCTGGT-3'		
14	DLD_EX14_F	5'-CCTGATGGTTACCACATGCA-3'	60ºC	405
	DLD_EX14_R	5'-TCTACTGAGGTAGCTTCCCCC-3'		

Table-4. Primers used for the DBT gene.

Ekzon	**Primers name**	**Primers**	t ºC	Amplicon size (b.p)
1	DBT_EX1_F	5'-CTTCCCTCCCTATTGGTCG-3'	60ºC	316
	DBT_EX1_R	5'-CTCCGTTCTCTGCCCTTTATT-3'		
2	DBT_EX2_F	5'-CAGAAGGAATTTTGGGTAAGGA-3'	62ºC	563
	DBT_EX2_R	5'-CAATCTGGGCAACTGAGTGA-3'		
3	DBT_EX3_F	5'-GCCTCTGCCTGAGAACATTC-3'	60ºC	376
	DBT_EX3_R	5'-TGTATGACAAAGTCCTTCACCACT-3'		
4	DBT_EX4_F	5'-CTGAAAGTAAATGCTGGGCTAGA-3'	60ºC	552
	DBT_EX4_R	5'-ATCTCTTCCTTTTGCTATTGCCT-3'		
5	DBT_EX5_F	5'-CCCACTCTACCCATACCATTAGG-3'	60ºC	606
	DBT_EX5_R	5'-AGCACCTGACATAAGACCTGGT-3'		
6	DBT_EX6_F	5'-CCTGATGGTTACCACATGCA-3'	60ºC	483
	DBT_EX6_R	5'-TCTACTGAGGTAGCTTCCCCC-3'		
7	DBT_EX7_F	5'-GCAGTCAGTGTTCCAGCTTTG-3'	60ºC	405
	DBT_EX7_R	5'-CAAATAAATGTCCTACTCAAGCCTT-3'		
8	DBT_EX8_F	5'-GGAACTTTGGCTGGTCTGTATC-3'	60ºC	660
	DBT_EX8_R	5'-GCTGCTTCTTTTTGAGAGGGT-3'		
9-10	DBT_EX9_10_F	5'-ATGGCAGTGAAGGTTGATCC-3'	60ºC	542
	DBT_EX9_10_R	5'-TGTGTTTAGTCCCTGAATTTGCT-3'		
11	DBT_EX11_F	5'-GGTTTGCCTGATCTTACACCA-3'	60ºC	469
	DBT_EX11_R	5'-CCCAGGAGAACCATTACACC-3'		