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Araştırma Makalesi -Research Article

Evaluation of SNPs and miRNAs in the *BID, MAPK10,* and *AGER* Genes Related to Alzheimer's Disease by Using *In Silico* Tools

Alzheimer Hastalığıyla İlişkili *BID, MAPK10* ve *AGER* Genlerindeki SNP ve miRNA'ların *In Silico* Araçlar Kullanılarak Değerlendirilmesi

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ABSTRACT

Alzheimer's disease (AD) is a multifactorial disease resulting from both genetic and environmental factors, which are pathologically defined by the accumulation of intracellular hyperphosphorylated tau protein, neurofibrils tangles, and extracellular amyloid β protein in the brain. The purpose of this study is to estimate the potentially damaging effects of missense single nucleotide polymorphisms (SNPs) in the *BID, MAPK10* and *AGER* genes associated with AD using various *in silico* tools and to determine the effects of SNPs on miRNAs. In addition, it is aimed to determine the gene-gene and protein-protein interactions through various software tools.

Consequently, it was estimated that there may be harmful effects of seven polymorphisms in the *BID* gene, twenty-seven in the *MAPK10* gene and three in the *AGER* gene. It was obtained that some SNPs decrease the effectiveness of miRNA-mRNA binding, enhance, break, create a new binding zone and/or destroy the miRNA-mRNA binding zone in the *BID* and MAPK10 genes. miRNA-SNP analyses could not provide information on the *AGER* gene.

In this study, SNPs in the *BID, MAPK10*, and *AGER* genes, which are estimated to be high-risk SNPs, will be able to provide data for future genotyping studies. SNPs that are estimated to be high-risk and SNPs that may have a role in miRNA- mRNA activity can be assessed as a priority in experimental studies related to AD.

In the future, experimental studies are proposed to investigate the clinical effects of harmful/disease-related missense SNPs and SNPs affecting mRNA-miRNA interaction.

Keywords- Alzheimer's Disease, AGER, BID, MAPK10, In silico

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ÖZ

Alzheimer hastalığı (AH), beyinde hücre içi hiperfosforile tau proteini, nörofibril yumakları ve hücre dışı amiloid β proteininin birikimi ile patolojik olarak tanımlanan hem genetik hem de çevresel faktörlerden kaynaklanan multifaktöriyel bir hastalıktır. Bu çalışmanın amacı, çeşitli *in silico* araçları kullanarak AH ile ilişkili *BID, MAPK10* ve *AGER* genlerindeki yanlış anlamlı tek nükleotid polimorfizmlerinin (SNP'ler) potansiyel olarak zarar verici etkilerini tahmin etmek ve SNP'lerin miRNA'lar üzerindeki etkilerini belirlemektir. Ayrıca çeşitli yazılım araçları ile gen-gen ve protein-protein etkileşimlerinin belirlenmesi amaçlanmaktadır.

Sonuç olarak, *BID* geninde yedi, *MAPK10* geninde yirmi yedi ve *AGER* geninde üç polimorfizmin zararlı etkilerinin olabileceği tahmin edilmiştir. *BID* ve *MAPK10* genlerinde bazı SNP'lerin miRNA-mRNA bağlanmasının etkinliğini azalttığı, arttırdığı, kırdığı, yeni bir bağlanma bölgesi oluşturduğu ve/veya miRNA-mRNA bağlama bölgesini yok ettiği elde edilmiştir. miRNA-SNP analizlerinde *AGER* genine ait bilgi edinilememiştir.

Bu çalışmada *BID, MAPK10 ve AGER* genlerindeki yüksek riskli olduğu tahmin edilen SNP'ler gelecekteki genotipleme çalışmaları için veri sağlayabilecektir. Yüksek riskli olduğu tahmin edilen SNP'ler ve miRNA-mRNA aktivitesinde rolü olabilecek SNP'ler AH ile ilgili deneysel çalışmalarda öncelikli olarak değerlendirilebilecektir. Gelecekte, zararlı/hastalıkla ilgili yanlış anlamlı SNP'lerin ve mRNA-miRNA etkileşimini etkileyen SNP'lerin klinik etkilerini araştırmak için deneysel çalışmalar önerilmektedir.

Anahtar Kelimeler- Alzheimer Hastalığı, AGER, BID, MAPK10, In silico

I.INTRODUCTION

Alzheimer's disease (AD) is a multifactorial disease resulting from both genetic and environmental factors, which are pathologically defined by the accumulation of intracellular hyperphosphorylated tau protein in the brain, and extracellular amyloid β protein. The genetic factors that cause AD began to be investigated in the early 1990s with linkage analysis methods [1].

The Human Genome Project and the technological methods that develop rapidly with it show that many diseases are not caused by a single gene, but are caused by many factors that occur in our DNA structure. By conducting many studies, such as genome-wide association studies (GWAS), many variations found simultaneously on the entire genome can be determined. As a result of these studies, many variations have been obtained in AD. With these studies, single nucleotide polymorphisms (SNP) and miRNAs found in genes have become the focus of attention [2].

SNP is defined as the single base pair positions formed by different sequence alleles that exist in healthy individual genomes within a given society [3]. The frequency of these alleles must be 1% or more in that society, alleles less than 1% are not considered SNP [4]. The SNP is divided into two mainly synonymous and non-synonymous groups. Non-synonymous is divided into two groups as missense and nonsense. Missense SNPs, on the other hand, cause nucleotide change, resulting in different amino acid coding [5]. SNPs facilitate the emergence of the disease by creating a predisposition or demonstrating a cumulative effect without the actual cause of the disease [6].

MicroRNAs (miRNAs) are RNA molecules that regulate mRNAs' expression, most of which do not encode proteins [7, 8] miRNAs exhibit tissue-specific expression differences. Most of the miRNAs discovered (such as miR-9, miR-128) are synthesized only in the brain [9, 10]. SNPs that may occur in the structures or target sites of miRNAs disrupt miRNA-mRNA coupling. SNPs affecting miRNA-mRNA interactions cause anomalies in protein levels. Therefore, miRNAs are able to disrupt molecular pathways such as neurogenesis, amyloid synthesis, and acquired immunity [9].

The aim of this study was to determine the SNPs within AD-related *BID, MAPK10*, and *AGER* genes and to estimate the potential damaging/harmful effects of missense SNPs on the proteins encoded by these genes using various *in silico* tools. In addition, it was aimed to identify the possible effects of SNPs in these genes on the miRNA-mRNA interactions using various bioinformatics tools. For this purpose, *BID, MAPK10*, and *AGER* genes were primarily selected and analyzed using bioinformatics tools in this study. *The BID* gene is involved in the apoptosis pathway and causes mitochondrial dysfunction [11]. *The MAPK10* gene is a member of the JNK family involved in the unfolded protein response signalling pathway and is specifically expressed in the brain and

manages neuronal processes by integrating biochemical signals [11]. *The AGER* gene causes the accumulation of Aß, which is the main cause of AD by being found in the AGE-RAGE signalling pathway [11].

II.MATERIALS AND METHODS

A series of tools and databases were used in the bioinformatics analysis in this study. The workflow was summarized in Figure 1.

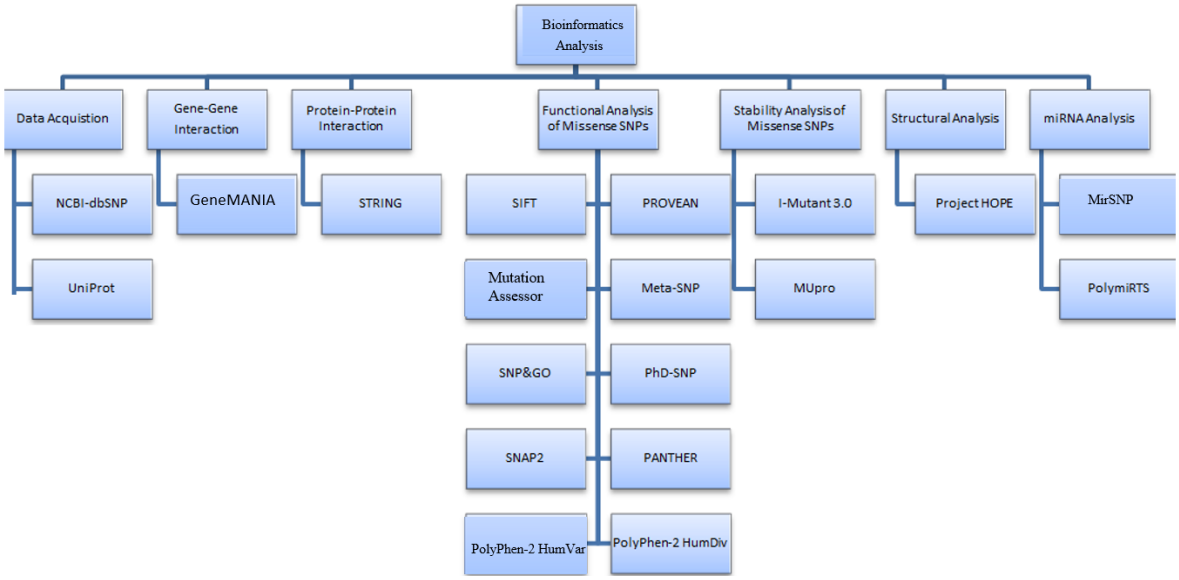


Figure 1. Workflow schema of bioinformatics analysis

A. Data Acquisition

The NCBI dbSNP database [12] was used to gain access to the missense SNPs of *BID* (NCBI Gene ID: 637), *MAPK10* (NCBI Gene ID: 5602), and *AGER* (NCBI Gene ID: 177) genes in December, February and March 2021, respectively. The missense SNPs within the *BID, MAPK10,* and *AGER* genes were chosen for bioinformatics analysis. The amino acid sequences of proteins encoded by the BID (UniProt accession number: P55957), MAPK10 (UniProt accession number: P53779) and AGER (UniProt accession number: Q15109) genes were obtained from the UniProt database [13].

B. Determination of gene-gene relationships

BID, MAPK10 and *AGER* genes' relationships with other genes were determined using the GeneMANIA [11] software tool. The GeneMANIA uses protein-protein interactions, protein-DNA interactions, gene-protein expression information, and phenotypic scanning data when creating gene-gene interaction data. In addition, it offers data by combining the associated gene networks with several different methods [14].

C. Determination of protein-protein relationships

BID, MAPK10 and AGER proteins' relationships with other proteins were determined using the STRING [15] software tool. The STRING offers protein-protein interactions functionally and physically. It creates an association study by compiling public texts and using computational methods [16].

D. In silico prediction of possible effects of SNPs

A series of online software tools have been used to identify harmful/damaging missense SNPs in the *BID, MAPK10* and *AGER* genes as described below:

Altering one of the amino acids that make up protein sequences can cause a change in structure and function in proteins. SIFT [17] (Sorting intolerant from tolerant) is a tool that analyses and estimates the physical properties and sequence homologies of amino acids to understand whether these changes actually cause changes in protein function [18, 19]. PolyPhen-2 [20] (Polymorphism phenotyping v2) estimates the effects of amino acid changes in protein using the protein's sequence, phylogenetic and structural properties. It classifies the estimate as damaging and benign according to the scoring system it uses. High scoring means that it is likely to affect the protein [21]. PROVEAN [22] is the software tool used to determine the possible effects of amino acid alterations on the protein function [23, 24]. The SNAP2 [25] is a neural network system that predicts whether amino acid change is harmful using the protein's structural properties and the effectiveness of the solvent in that protein [26]. The SNPs&GO [27] is a machine learning method that estimates whether altered amino acids are associated with

the disease, taking into account the function of proteins. It provides forecast results using the functional scores it generates [28]. PhD-SNP [29] (Predictor of human deleterious single nucleotide polymorphisms) is machine learning that estimates whether amino acid change is associated with the disease, using multiple-array alignment protection scores [30]. Mutation Assessor [31] is a tool that estimates the functional effects of mutations discovered in missense polymorphisms or cancer disease on protein. In order to determine the functional effect, the program scores based on the evolutionary preservation of the affected amino acid in multiple-array alignment [32]. PANTHER [33] (Protein analysis through evolutionary relationships) is a comprehensive software tool that estimates whether SNP's formed in a protein sequence are harmful to protein structure, taking into account the evolutionary histories and functions [34]. META-SNP [35] is a complex software tool that presents whether amino acid changes in the protein sequence are associated with the disease. It uses SIFT, PANTHER, SNAP2, and PhD-SNP software to generate analysis results [36].

E. Estimation of Protein Stabilization Change

I-Mutant 3.0 and MUpro were used for the estimation of protein stabilization change. I-Mutant 3.0 [37] is a tool used to estimate the impact of amino acid changes in the protein sequence on protein stability. It also uses DeltaDeltaG (free energy change) to generate the forecast result [38]. MUpro [39] is a neural network system that estimates the effects of non-synonymous amino acid alterations on protein stability using DeltaDeltaG score [40].

F. Determination of amino acid properties and models

Project HOPE [41] tool used in this study creates 3-D models of the proteins and compares amino acid changes caused by SNPs in terms of size, charge and hydrophobicity [42].

G. miRNA analysis of BID, MAPK10 and AGER genes

MirSNP and PolymiRTS software tools were used to identify SNPs that affected miRNAs, which regulate gene expression. The MirSNP [43] is a tool that predicts the effects of SNPs located in the 3'-UTR region on mRNA-miRNA binding. Those SNPs may have an influence on the miRNA-mRNA binding as an enhance, break, create and decrease[44]. PolymiRTS [45] provides information on SNPs occurring in miRNA seed regions and their target sites. The database shows the effect of SNPs in miRNA seed regions under functional classes as D, C, N and O. D: The derived allele disrupts a protected miRNA region. C: The derived allele creates a new miRNA region. N: The derived allele disrupts a nonconserved miRNA site (ancestral allele with support < 2). O: Refers to situations that the ancestral allele cannot be determined [46].

III. RESULTS

SNPs of human *BID, MAPK10, and AGER* genes were procured from the NCBI dbSNP. *BID* gene was determined to contain a total of 12440 SNPs of which 186 SNPs were missense. There was a total of 132004 SNPs found in the *MAPK10* gene of which 204 SNPs were missense. Finally, the *AGER* gene was determined to comprise a total of 1840 SNPs. 392 SNPs among them were missense. Those missense SNPs were selected for further analysis.

A. Results of gene-gene relationships

The relationships of *BID, MAPK10, and AGER* genes with other genes were shown in Figure 2A, Figure 2B, and Figure 2C, respectively. The 5 genes with which the *BID* gene interacts most closely: *BAK1*, *BCL2*, *BAX*, *BCL2L1*, and *NMT1*. When the input was limited to 20 genes, 279 connections were determined between them (Figure 2A). The 5 genes with which the *MAPK10* gene interacts most closely: *EBF1*, *SARM1*, *ARRB2*, *MAPK8IP3*, and *JUND*. When the input was limited to 20 genes, 143 connections were determined between them (Figure 2B). The 5 genes with which the *AGER* gene interacts most closely: *RELA*, *HMGB1*, *ITGAM*, *S100P*, and *S100A12*. When the input was limited to 20 genes, 262 connections were determined between them (Figure 2C).

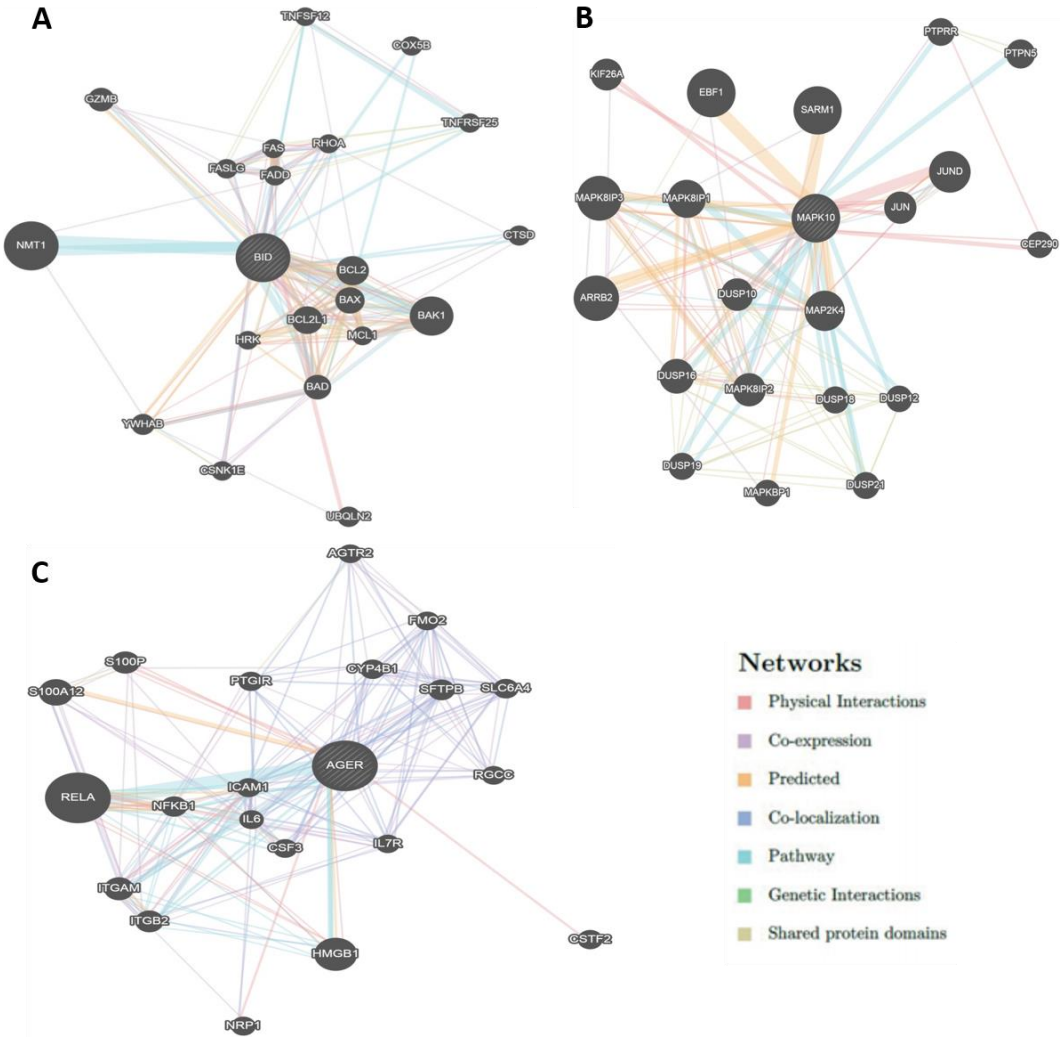


Figure 2. The interactions between genes related to the (A) *BID* gene (B) *MAPK10* gene, (C) *AGER* gene from GeneMANIA [11].

B. Results of protein-protein relationships

The input of the STRING software was limited to ten proteins to obtain protein-protein interactions of BID, MAPK10, and AGER proteins. As a result, 48, 30, and 41 connections were determined for BID, MAPK10, and AGER proteins, respectively (Figure 3).

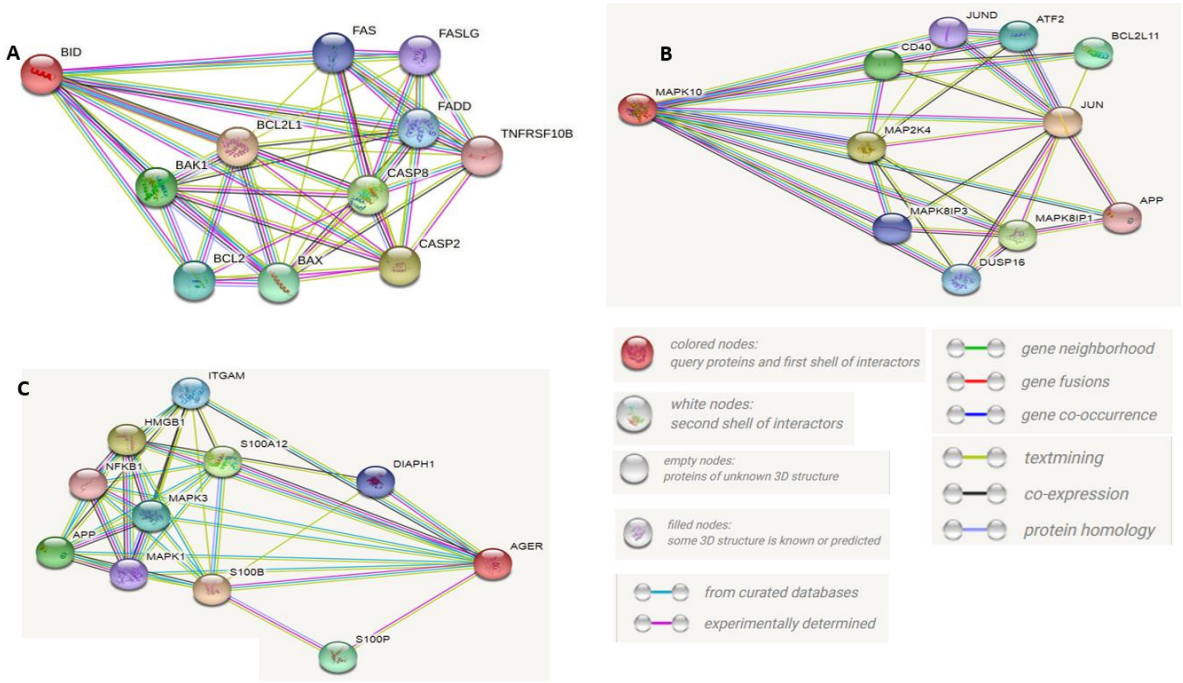


Figure 3. The protein-protein interactions of (A) BID, (B) MAPK10, (C) AGER and the types of connections from STRING [15]

C. In silico prediction results of possible effects of SNPs

The missense variants of *BID*, *MAPK10*, and *AGER* genes were analyzed via SIFT, PolyPhen-2 (HumVar and HumDiv), PROVEAN, Mutation Assessor, SNPs&GO, PhD-SNP, PANTHER, SNAP2, and META-SNP software tools. 7 disease-related/harmful SNPs were identified in the *BID* gene (out of 10 data from 9 software tools), 27 in the *MAPK10* gene (Out of 9 data from 8 software tools), and 3 in the *AGER* gene (out of 10 data from 9 software tools). The results are shown in Table 1-2 for *BID*, *MAPK10*, and *AGER* genes, respectively.

Table 1. Functional analysis results of SNPs from SIFT, PolyPhen-2, PROVEAN, and PANTHER

Gene	SNP ID	A.A.	SIFT	SIFT Score	PolyPhen-2 HumDiv	PolyPhen-2 HumDiv Score	PolyPhen-2 HumVar	PolyPhen-2 HumVar Score	PROVEAN	PROVEAN Score	PANTHER
BID	rs113033070	L19P	D.	0	PrD	1	PrD	0,998	D.	-4,531	PrD
BID	rs143734092	L151P	D.	0,005	PrD	0,999	PrD	0,99	D.	-5,964	Not found
BID	rs143734092	L105P	D.	0,007	PrD	1	PrD	0.983	D.	-5,071	PrD
BID	rs367829996	N181K	D.	0,013	PrD	0,999	PrD	0,978	D.	-4,506	PrD
BID	rs376912423	F171V	D.	0,006	PrD	1	PrD	0,998	D.	-5,714	Not found
BID	rs780833600	G8V	Not found	Not found	PrD	0,999	PrD	0,987	D.	-4,473	Not found
BID	rs1196785035	G8D	Not found	Not found	PrD	0,999	PrD	0.989	D.	-3,427	PrD
MAPK10	rs142571603	W390R	D.	0	PrD	1	PrD	1	D.	-13,13	Not found
MAPK10	rs143720396	A91V	Not found	Not found	PrD	0,998	PrD	0,944	D.	-3,334	Not found
MAPK10	rs372124619	W272R	D.	0,002	PrD	1	PrD	1	D.	-13,329	Not found
MAPK10	rs376073317	Y268C	D.	0,039	PrD	0,962	PrD	0,861	D.	-5,11	Not found
MAPK10	rs534048937	D189Y	Not found	Not found	PrD	1	PrD	1	D.	-8,368	Not found
MAPK10	rs756256876	N122Y	Not found	Not found	PrD	1	PrD	1	D.	-7,084	Not found
MAPK10	rs757457303	L153F	Not found	Not found	PrD	1	PrD	1	D.	-3,849	Not found
MAPK10	rs762664125	D207N	Not found	Not found	PrD	1	PrD	0,998	D.	-4,668	Not found
MAPK10	rs773390425	R63C	Not found	Not found	PrD	1	PrD	0,999	D.	-7,267	Not found
MAPK10	rs777561999	L190F	Not found	Not found	PrD	1	PrD	0,999	D.	-3,728	Not found
MAPK10	rs778824606	A231T	Not found	Not found	PrD	1	PrD	0.994	D.	-3,604	Not found
MAPK10	rs937510593	G250R	Not found	Not found	PrD	1	PrD	1	D.	-7,083	Not found
MAPK10	rs939639515	V244L	Not found	Not found	PoD	0,901	PoD	0,714	D.	-2,582	Not found
MAPK10	rs951209020	L95P	Not found	Not found	PrD	1	PrD	0.999	D.	-6,078	Not found
MAPK10	rs1047724692	V53G	Not found	Not found	PrD	0,989	PrD	0.959	D.	-5,892	Not found
MAPK10	rs1200724232	M339I	Not found	Not found	PrD	1	PrD	1	D.	-3,713	Not found
MAPK10	rs1219541757	G237E	Not found	Not found	PoD	0.932	PoD	0,817	D.	-7,423	Not found
MAPK10	rs1241029338	C251Y	Not found	Not found	PrD	1	PrD	1	D.	-9,858	Not found
MAPK10	rs1304596003	D141H	Not found	Not found	PrD	0.989	PrD	0,911	D.	-5,596	Not found
MAPK10	rs1314239624	R188M	Not found	Not found	PrD	1	PrD	0,998	D.	-5,525	Not found
MAPK10	rs1337478269	A353D	Not found	Not found	PrD	1	PrD	1	D.	-5,472	Not found
MAPK10	rs1336009977	V145L	Not found	Not found	PrD	0.968	PoD	0.865	D.	-2,792	Not found
MAPK10	rs1367894037	C201W	Not found	Not found	PrD	1	PrD	1	D.	-10,43	Not found
MAPK10	rs1580038340	W247R	Not found	Not found	PrD	1	PrD	1	D.	-12,594	Not found
MAPK10	rs939639515	V244M	Not found	Not found	PrD	0.998	PoD	0,886	D.	-2,652	Not found
MAPK10	rs1255789663	L190S	Not found	Not found	PrD	1	PrD	0,998	D.	-5,559	Not found
MAPK10	rs373516870	E255Q	Not found	Not found	PrD	0.998	PrD	0.991	D.	-2.742	Not found
AGER	rs138178120	C301S	D.	0	PrD	1	PrD	0,999	D.	-6,661	PrD
AGER	rs138726985	C144W	D.	0	PrD	1	PrD	1	D.	-9,74	PrD
AGER	rs201829223	C38W	D.	0	PrD	1	PrD	1	D.	-7.217	PrD

D: Deleterious; PrD: Probably Damaging, PoD: Possibly damaging

Table 2. Functional analysis results of SNPs from SNP&GO, Mutation Assessor, SNAP-2, PHD-SNP, and META-SNP

Gene	SNP ID	A.A.	SNPs&GO	SNPs&GO Score	Mutation Assessor	Mutation Assessor FI. Score	SNAP-2	SNAP-2 Score	SNAP-2 Expected Accuracy	PHD-SNP	PHD-SNP RI Score	META-SNP
BID	rs113033070	L19P	Disease	6	Medium	2,165	Effect	79	85%	Disease	8	Disease
BID	rs143734092	L151P	Disease	2	Medium	1,975	Effect	71	85%	Disease	4	Disease
BID	rs143734092	L105P	Disease	2	Low	1,935	Effect	83	91%	Disease	2	Disease
BID	rs367829996	N181K	Disease	5	Medium	2,14	Effect	61	80%	Disease	6	Disease
BID	rs376912423	F171V	Disease	0	Medium	2,14	Effect	69	80%	Disease	4	Disease
BID	rs780833600	G8V	Disease	2	Medium	2,165	Effect	60	80%	Disease	4	Disease
BID	rs1196785035	G8D	Disease	2	Medium	2,165	Effect	73	85%	Disease	1	Disease
MAPK10	rs142571603	W390R	Neutral	5	Medium	2,36	Effect	73	85%	Disease	0	Disease
MAPK10	rs143720396	A91V	Disease	6	Medium	2,375	Effect	30	66%	Disease	6	Disease
MAPK10	rs372124619	W272R	Disease	7	Neutral	0,58	Effect	74	85%	Disease	7	Disease
MAPK10	rs376073317	Y268C	Neutral	6	Low	1,68	Effect	38	66%	Disease	1	Disease
MAPK10	rs534048937	D189Y	Disease	3	High	4,4	Effect	94	95%	Disease	9	Disease
MAPK10	rs756256876	N122Y	Disease	6	Medium	2,42	Effect	43	71%	Disease	7	Disease
MAPK10	rs757457303	L153F	Disease	3	Medium	2,58	Effect	79	85%	Disease	7	Disease
MAPK10	rs762664125	D207N	Disease	4	Medium	3,42	Effect	90	95%	Disease	6	Disease
MAPK10	rs773390425	R63C	Disease	3	Low	0,895	Effect	25	63%	Disease	6	Disease
MAPK10	rs777561999	L190F	Disease	1	Medium	2,98	Effect	65	80%	Disease	5	Disease
MAPK10	rs778824606	A231T	Disease	3	High	3,605	Effect	51	75%	Disease	2	Disease
MAPK10	rs937510593	G250R	Disease	6	High	3,885	Effect	92	95%	Disease	9	Disease
MAPK10	rs939639515	V244L	Disease	0	Medium	2,17	Effect	85	91%	Disease	6	Disease
MAPK10	rs951209020	L95P	Neutral	6	Low	2,145	Effect	73	85%	Disease	7	Disease
MAPK10	rs1047724692	V53G	Neutral	2	Low	0,805	Effect	76	85%	Disease	8	Disease
MAPK10	rs1200724232	M339I	Disease	4	Medium	1,355	Effect	83	91%	Disease	5	Disease
MAPK10	rs1219541757	G237E	Disease	4	Medium	3,015	Effect	95	95%	Disease	7	Disease
MAPK10	rs1241029338	C251Y	Disease	8	Medium	1,115	Effect	45	71%	Disease	9	Disease
MAPK10	rs1304596003	D141H	Neutral	4	Low	3,605	Effect	65	80%	Disease	7	Disease
MAPK10	rs1314239624	R188M	Disease	4	Medium	3,37	Effect	85	91%	Disease	6	Disease
MAPK10	rs1337478269	A353D	Disease	3	High	4,045	Effect	73	85%	Disease	8	Disease
MAPK10	rs1336009977	V145L	Disease	0	Low	1,745	Effect	76	85%	Disease	2	Disease
MAPK10	rs1367894037	C201W	Disease	5	Low	1,44	Effect	83	91%	Disease	0	Disease
MAPK10	rs1580038340	W247R	Disease	8	High	4,635	Effect	95	95%	Disease	9	Disease
MAPK10	rs939639515	V244M	Disease	1	Medium	2,47	Effect	45	71%	Disease	8	Disease
MAPK10	rs1255789663	L190S	Disease	5	High	3,875	Effect	65	80%	Disease	6	Disease
MAPK10	rs373516870	E255Q	Disease	5	Medium	2.225	Effect	83	91%	Disease	3	Disease
AGER	rs138178120	C301S	Disease	2	High	4,235	Effect	76	85%	Disease	5	Disease
AGER	rs138726985	C144W	Disease	5	Medium	3,33	Effect	84	91%	Disease	7	Disease
AGER	rs201829223	C38W	Disease	2	Medium	3.385	Effect	85	91%	Disease	8	Disease

D. Protein Stabilization Results

The deleterious SNPs that were predicted by *in silico* tools as described in Section C were analyzed via I-Mutant and MUpro software tools. All of those amino acid changes caused by SNPs were predicted to decrease protein stability as shown in Table 3.

Table 3. Prediction results of protein stability

Gene	SNP ID	Nucleotide Change	Amino Acid Change	I-Mutant 3.0 Prediction	I-Mutant 3.0 RI	I-Mutant 3.0 DDG	MUpro Prediction	MUpro DDG
BID	rs113033070	A>G	L19P	Decrease	4	-1.65	Decrease	-2.3128234
BID	rs143734092	A>G	L151P	Decrease	6	-1.15	Decrease	-12.247.708
BID	rs143734092	A>G	L105P	Decrease	3	-1.71	Decrease	-20.025.928
BID	rs367829996	G>T	N181K	Decrease	6	-0.62	Decrease	-1.398417
BID	rs376912423	A>C	F171V	Decrease	8	-1.69	Decrease	-1.420.371
BID	rs780833600	C>A	G8V	Decrease	8	-0.71	Decrease	-0.4017768
BID	rs1196785035	C>T	G8D	Decrease	9	-1.20	Decrease	-0.66696436
MAPK10	rs142571603	A>G	W390R	Decrease	6	-0.62	Decrease	-1.0713584
MAPK10	rs143720396	G>A / G>C	A91V	Decrease	4	-0.39	Decrease	-0.58308152
MAPK10	rs372124619	A>G	W272R	Decrease	8	-1.16	Decrease	-15.438.938
MAPK10	rs373516870	C>G	E255Q	Decrease	7	-0.40	Decrease	-0.73364885
MAPK10	rs376073317	T>C	Y268C	Decrease	2	-1.10	Decrease	-0.76248657
MAPK10	rs534048937	C>A	D189Y	Decrease	3	-0.48	Decrease	-1.0071177
MAPK10	rs756256876	T>A	N122Y	Decrease	0	-0.07	Decrease	-0.73065093
MAPK10	rs757457303	T>C / T>G	L153F	Decrease	5	-0.92	Decrease	-0.80002274
MAPK10	rs762664125	C>T	D207N	Decrease	5	-0.64	Decrease	-0.92204047
MAPK10	rs773390425	G>A	R63C	Decrease	4	-1.19	Decrease	-0.7351192
MAPK10	rs777561999	C>A / C>T	L190F	Decrease	7	-1.14	Decrease	-1.245821
MAPK10	rs778824606	C>T	A231T	Decrease	6	-0.68	Decrease	-1.0137092
MAPK10	rs937510593	C>G	G250R	Decrease	4	-0.48	Decrease	-0.38427059
MAPK10	rs939639515	C>A / C>T	V244L	Decrease	2	-0.87	Decrease	-0.75885538
MAPK10	rs951209020	A>G	L95P	Decrease	7	-1.91	Decrease	-15.943.355
MAPK10	rs1047724692	A>C	V53G	Decrease	10	-2.86	Decrease	-2.725322
MAPK10	rs1200724232	C>T	M339I	Decrease	5	-0.61	Decrease	-0.95105635
MAPK10	rs1219541757	C>T	G237E	Decrease	6	-0.86	Decrease	-0.54729308
MAPK10	rs1241029338	C>T	C251Y	Decrease	1	-0.33	Decrease	-11.912.536
MAPK10	rs1304596003	C>G	D141H	Decrease	3	-0.33	Decrease	-0.8588084
MAPK10	rs1314239624	C>A	R188M	Decrease	5	-0.57	Decrease	-0.40248524
MAPK10	rs1337478269	G>T	A353D	Decrease	6	-0.72	Decrease	-0.96748389
MAPK10	rs1336009977	C>A / C>T	V145L	Decrease	6	-1.06	Decrease	-0.51623757
MAPK10	rs1367894037	G>C	C201W	Decrease	2	-0.26	Decrease	-11.970.366
MAPK10	rs1580038340	A>G	W247R	Decrease	7	-0.91	Decrease	-15.666.698
MAPK10	rs939639515	C>A / C>T	V244M	Decrease	5	-0.89	Decrease	-0.91713103
MAPK10	rs1255789663	A>G	L190S	Decrease	8	-2.12	Decrease	-1.9851829
AGER	rs138178120	C>G / C>T	C301S	Decrease	5	-0.63	Decrease	-1.3749336
AGER	rs138726985	A>C	C144W	Decrease	2	-0.21	Decrease	-0.67606746
AGER	rs201829223	A>C	C38W	Decrease	1	0.04	Decrease	-1.244.702

DDG: DeltaDelta G RI: Reliability Index

E. Results of amino acid features and modeling via Project HOPE

The features of variant residues such as size, charge, and hydrophobicity were summarized in Table 4-6 and three-dimensional models of BID, MAPK10 and AGER were given in Table 7-9, respectively. In addition, interactions of wild type residues with other residues in the MAPK10 protein and possible effects of variations on these interactions are presented in Table 10.

Table 4. Properties of wild and variant type amino acids of BID from Project HOPE [42]

		WILD-TYPE AMINO ACIDS			MUTANT TYPE AMINO ACIDS		
SNP ID	**Amino Acid Change**	**Size**	**Charge**	**Hydrophobicity**	**Size**	**Charge**	**Hydrophobicity**
rs113033070	L19P	>	-	-	<	-	-
rs143734092	L151P	>	-	-	<	-	-
rs143734092	L105P	>	-	-	<	-	-
rs367829996	N181K	<	Neutral	-	>	+ Charge	-
rs376912423	F171V	>	-	-	<	-	-
rs780833600	G8V	<	-	<	>	-	>
rs1196785035	G8D	<	Neutral	>	>	- Charge	<

Table 5. Properties of wild and variant type amino acids of MAPK10 from Project HOPE [42]

		WILD-TYPE AMINO ACIDS			MUTANT TYPE AMINO ACIDS		
SNP ID	**Amino Acid Change**	**Size**	**Charge**	**Hydrophobicity**	**Size**	**Charge**	**Hydrophobicity**
rs142571603	W390R	>	Neutral	>	<	+ Charge	<
rs143720396	A91V	<	-	-	>	-	-
rs372124619	W272R	>	Neutral	>	<	+ Charge	<
rs373516870	E255Q	-	- Charge	-	-	Neutral	-
rs376073317	Y268C	>	-	<	<	-	>
rs534048937	D189Y	<	- Charge	<	>	Neutral	>
rs756256876	N122Y	<	-	<	>	-	>
rs757457303	L153F	<	-	-	>	-	-
rs762664125	D207N	-	- Charge	-	-	Neutral	-
rs773390425	R63C	<	+ Charge	<	>	Neutral	>
rs777561999	L190F	<	-	-	>	-	-
rs778824606	A231T	<	-	>	>	-	<
rs937510593	G250R	<	Neutral	>	>	+ Charge	<
rs939639515	V244L	<	-	-	>	-	-
rs951209020	L95P	>	-	-	<	-	-
rs1047724692	V53G	>	-	>	<	-	<
rs1200724232	M339I	>	-	-	<	-	-
rs1219541757	G237E	<	Neutral	>	>	- Charge	<
rs1241029338	C251Y	<	-	>	>	-	<
rs1304596003	D141H	<	- Charge	-	>	Neutral	-
rs1314239624	R188M	>	+ Charge	<	<	Neutral	>
rs1337478269	A353D	<	Neutral	>	>	- Charge	<
rs1336009977	V145L	<	-	-	>	-	-
rs1367894037	C201W	<	-	-	>	-	-
rs1580038340	W247R	>	Neutral	>	<	+ Charge	<
rs939639515	V244M	<	-	-	>	-	-
rs1255789663	L190S	>	-	>	<	-	<

Table 6. Properties of wild and variant type amino acids of AGER from Project HOPE [42]

		WILD-TYPE AMINO ACIDS			MUTANT TYPE AMINO ACIDS		
SNP ID	**Amino Acid Change**	**Size**	**Charge**	**Hydrophobicity**	**Size**	**Charge**	**Hydrophobicity**
rs138178120	C301S	-	-	>	-	-	<
rs138726985	C144W	<	-	-	>	-	-
rs201829223	C38W	<	-	-	>	-	-

Table 7. Results of the 3D models of the BID protein via Project HOPE [42]

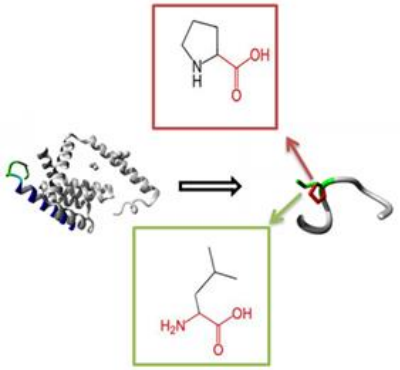
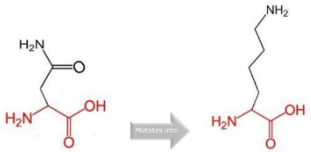
rs113033070 **L19P**	**rs143734092** **L151P**	**rs143734092** **L105P**	**rs367829996** **N181K**
			
rs376912423 **F171V**	**rs780833600** **G8V**	**rs1196785035** **G8D**	
			

Table 8. Results of the 3D models of the MAPK10 protein via Project Hope [42]

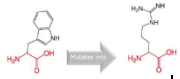
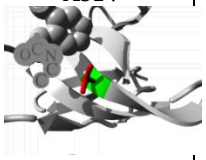
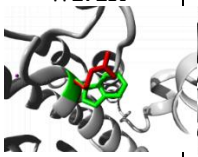
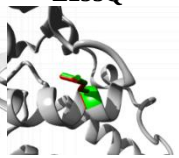
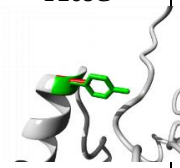
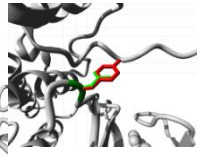
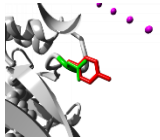
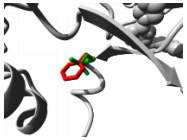
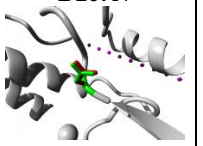
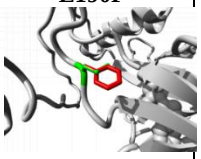
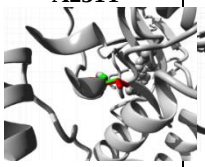
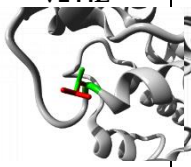
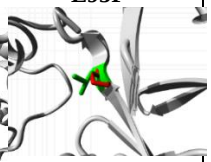
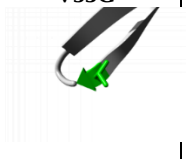
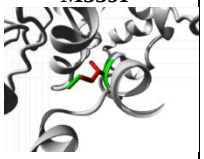
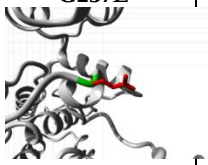
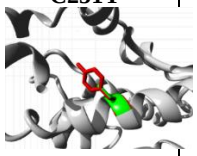
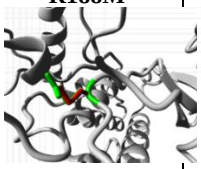
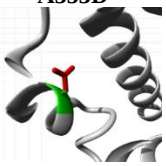
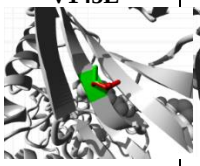
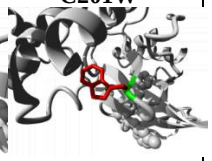
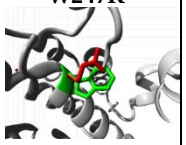
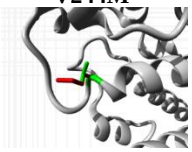
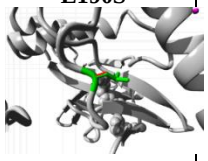
rs142571603 W390R 	**rs143720396 A91V** 	**rs372124619 W272R** 	**rs373516870 E255Q** 	**rs376073317 Y268C** 	**rs534048937 D189Y** 	**rs756256876 N122Y** 	**rs757457303 L153F** 	**rs762664125 D207N** 
rs773390425 R63C 	**rs777561999 L190F** 	**rs778824606 A231T** 	**rs937510593 G250R** 	**rs939639515 V244L** 	**rs951209020 L95P** 	**rs1047724692 V53G** 	**rs1200724232 M339I** 	**rs1219541757 G237E** 
rs1241029338 C251Y 	**rs1304596003 D141H** 	**rs1314239624 R188M** 	**rs1337478269 A353D** 	**rs1336009977 V145L** 	**rs1367894037 C201W** 	**rs1580038340 W247R** 	**rs939639515 V244M** 	**rs1255789663 L190S** 

Table 9. Results of the 3D models of the AGER protein via Project Hope [42]

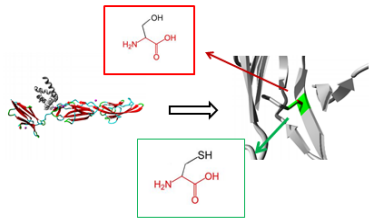
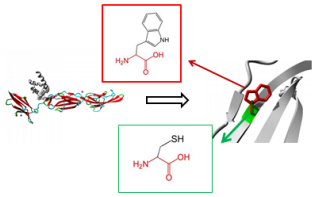
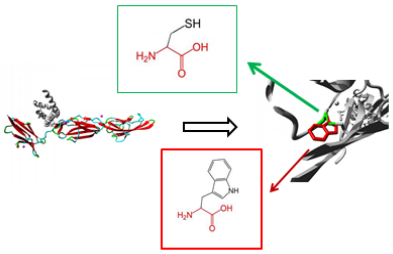
rs138178120 C301S	rs138726985 C144W	rs201829223 C38W
		

Table 10. Project HOPE software results of interactions by MAPK10 variants [42]

SNP ID	Amino Acid Change	Types of Bonds Established by Wild Type Amino Acid	Effect of Variation on Protein
rs773390425	R63C	It forms a hydrogen bond with aspartic acid in position 83 and forms a salt bridge.	As a result of the variation, these types of interaction may be affected due to the change in amino acid size, load, and hydrophobicity.
rs1304596003	D141H	It forms a salt bridge with lysine in the 94th position and arginine in the 97th position.	The difference in load as a result of the variation is thought to disrupt ionic interactions.
rs756256876	N122Y	It establishes hydrogen bonds with histidine in the 120th position and leucine in the 203rd position.	Differences in size and hydrophobicity that will occur in amino acids as a result of the variation can lead to deterioration of hydrogen bonds.
rs376073317	Y268C	In the 230th position, it establishes a hydrogen bond with arginine.	It causes the new position not to be in the right position to make the same hydrogen bond as the original wild-type position did. Hydrogen bond formation can be affected by hydrophobic change.
rs534048937	D189Y	It establishes hydrogen bonds with arginine in the 230th position and lysine in the 191st position. It also forms a salt bridge with arginine in the 107th and 110th positions and lysine in the 191st position.	Changing hydrophobicity can disrupt these interactions. Due to the load difference, this can disrupt ionic connections.
rs762664125	D207N	It establishes hydrogen bonds with histidine at the 187th position. It forms salt bridges with arginine in the 107th and 110th positions and lysine in the 191st positions.	The load difference as a result of the variation can disrupt such ionic connections.
rs1314239624	R188M	It forms salt bridges with aspartic acid in the 245th position and glutamic acid in the 382nd position.	The load change resulting from the variation can disrupt these ionic interactions.

F. miRNA Analysis Results Associated with BID, MAPK10, and AGER Genes

MirSNP and PolymiRTS software tools were used to analyze SNPs that are in *BID*, *MAPK10*, and *AGER* genes and cause various effects at miRNA-mRNA binding sites. The results of *BID* and *MAPK10* genes were presented in Table 11 and Table 12, respectively. There are no results were obtained for the *AGER* gene with both MirSNP and PolymiRTS.

Table 11. SNPs and miRNAs in *BID* gene predicted by miRNA target prediction databases.

Gene	Variation	dbSNP ID	miRNA	MirSNP	PolymiRTS	MirSNP Effect	PolymiRTS Effect
BID	A/C	rs1061200	hsa-miR-3145-3p	+	-	decrease	
BID	A/C	rs1061200	hsa-miR-4796-3p	+	-	break	
BID	T/A	rs1802194	hsa-miR-4289	-	+		C
BID	T/C	rs186394629	hsa-miR-141-5p	-	+		D
BID	T/C	rs186394629	hsa-miR-626	-	+		C
BID	T/C	rs186394629	hsa-miR-6740-3p	-	+		C
BID	T/C	rs186394629	hsa-miR-6876-3p	-	+		C
BID	A/T	rs1802194	hsa-miR-302c-5p	+	-	create	
BID	A/T	rs1802194	hsa-miR-3143	+	-	create	
BID	A/T	rs1802194	hsa-miR-4796-3p	+	-	enhance	
BID	T/A	rs2305001	hsa-miR-3121-3p	+	-	create	
BID	G/A	rs2305001	hsa-miR-3121-3p	+	-	create	
BID	G/A	rs2305001	hsa-miR-3143	+	-	decrease	
BID	G/A	rs2305001	hsa-miR-3143	+	-	decrease	
BID	T/G	rs2305001	hsa-miR-511	+	-	enhance	
BID	A/A	rs8190355	hsa-miR-4326	-	+		D
BID	A/G	rs8190355	hsa-miR-567	+	+	break	
BID	A/A	rs8190355	hsa-miR-567	+	+		D
BID	A/A	rs8190355	hsa-miR-6868-3p	-	+		D
BID	A/G	rs8190355	hsa-miR-3936	+	-	decrease	
BID	A/G	rs8190355	hsa-miR-4448	+	-	create	
BID	-/ATCT	rs8190356	hsa-miR-4477a	+	-	enhance	
BID	-/A	rs8190357	hsa-miR-4659a-5p	+	-	decrease	
BID	A/A	rs8190358	hsa-miR-6507-3p	-	+		D
BID	T/T	rs151268390	hsa-miR-6507-3p	-	+		D
BID	T/G	rs151268390	hsa-miR-4680-3p	+	-	decrease	
BID	-/CA	rs71680500	hsa-miR-3120-3p	+	-	decrease	
BID	G/G	rs115649437	hsa-miR-1266-3p	-	+		D
BID	G/G	rs115649437	hsa-miR-452-5p	-	+		D
BID	G/G	rs115649437	hsa-miR-3074-5p	-	+		D
BID	G/T	rs115649437	hsa-miR-7152-5p	-	+		C
BID	G/T	rs115649437	hsa-miR-892c-3p	-	+		C
BID	G/T	rs115649437	hsa-miR-4693-5p	-	+		C
BID	G/T	rs115649437	hsa-miR-4676-3p	-	+		C
BID	T/G	rs115649437	hsa-miR-513a-5p	+	-	enhance	
BID	T/T	rs151268390	hsa-miR-6507-3p	-	+		D

Table 11. *(Continued)*

BID	T/G	rs151268390	hsa-miR-302c-5p	-	+		C
BID	T/G	rs151268390	hsa-miR-552-5p	-	+		C
BID	T/G	rs151268390	hsa-miR-6882-5p	-	+		C
BID	T/G	rs151268390	hsa-miR-4680-3p	+	-	decrease	
BID	G/A	rs189671157	hsa-miR-130b-5p	+	+	create	
BID	A/A	rs189671157	hsa-miR-130b-5p	+	+		D
BID	G/A	rs189671157	hsa-miR-18a-3p	+	+	Break	
BID	A/G	rs189671157	hsa-miR-18a-3p	+	+		C
BID	A/G	rs189671157	hsa-miR-6886-3p	-	+		C
BID	A/G	rs189671157	hsa-miR-938	-	+		C
BID	G/A	rs192534350	hsa-miR-130b-5p	+	+	create	
BID	A/A	rs192534350	hsa-miR-130b-5p	+	+		D
BID	A/G	rs192534350	hsa-miR-6870-3p	-	+		C
BID	A/G	rs192534350	hsa-miR-7110-3p	-	+		C
BID	A/A	rs192534350	hsa-miR-452-3p	-	+		D
BID	A/A	rs189629146	hsa-miR-29a-5p	-	+		D
BID	A/C	rs189629146	hsa-miR-3613-5p	-	+		C
BID	C/A	rs149980557	hsa-miR-3661	+	+	break	
BID	C/C	rs149980557	hsa-miR-3661	+	+		D
BID	C/A	rs149980557	hsa-miR-619-3p	-	+		C
BID	C/A	rs149980557	hsa-miR-631	+	+	break	
BID	C/C	rs149980557	hsa-miR-631	+	+		D
BID	C/A	rs149980557	hsa-miR-7851-3p	-	+		C
BID	C/A	rs149980557	hsa-miR-30a-5p	+	-	decrease	
BID	C/A	rs149980557	hsa-miR-30d-5p	+	-	decrease	
BID	C/A	rs149980557	hsa-miR-30e-5p	+	-	decrease	
BID	C/A	rs149980557	hsa-miR-5693	+	-	decrease	
BID	G/A	rs147656401	hsa-miR-145-3p	-	+		C
BID	G/A	rs147656401	hsa-miR-3145-5p	+	-	break	
BID	T/A T/T	rs146895750	hsa-miR-4775	+	+	break	
BID	T/A T/T	rs146895750	hsa-miR-4775	+	+		D
BID	T/A	rs146895750	hsa-miR-144-3p	+	+	create	
BID	T/A	rs146895750	hsa-miR-144-3p	+	+		C
BID	T/T	rs146895750	hsa-miR-590-3p	-	+		D
BID	T/A	rs146895750	hsa-miR-16-1-3p	+	+	create	
BID	T/A	rs146895750	hsa-miR-16-1-3p	+	+		C
BID	C/T	rs181869684	hsa-miR-511-3p	-	+		C
BID	T/C	rs181869684	hsa-miR-124-3p	+	-	enhance	

Table 11. *(Continued)*

BID	T/C	rs181869684	hsa-miR-548an	+	-	enhance	
BID	T/C	rs181869684	hsa-miR-593-5p	+	-	break	
BID	T/C	rs181869684	hsa-miR-767-5p	+	-	break	
BID	T/C T/T	rs186394629	hsa-miR-141-5p	+	+	break	
BID	T/C T/T	rs186394629	hsa-miR-141-5p	+	+		D
BID	T/C	rs186394629	hsa-miR-626	+	+	create	
BID	T/C	rs186394629	hsa-miR-626	+	+		C
BID	T/C	rs186394629	hsa-miR-6740-3p	-	+		C
BID	T/C	rs186394629	hsa-miR-6876-3p	-	+		C
BID	T/C	rs186394629	hsa-miR-224-5p	+	-	enhance	
BID	T/C	rs148682143	hsa-miR-1299	+	-	enhance	
BID	G/C	rs113283699	hsa-miR-1303	+	-	create	
BID	G/C	rs113283699	hsa-miR-5683	+	-	break	
BID	-/CTTT	rs8190359	hsa-miR-3133	+	-	create	
BID	-/CTTT	rs8190359	hsa-miR-4773	+	-	create	
BID	-/CTTT	rs78336589	hsa-miR-3133	+	-	create	
BID	-/CTTT	rs78336589	hsa-miR-4773	+	-	create	
BID	C/T	rs8190361	hsa-miR-3177-3p	+	-	create	
BID	C/T	rs8190361	hsa-miR-3677-5p	+	-	enhance	
BID	C/T	rs8190361	hsa-miR-4479	+	-	break	
BID	C/T	rs8190361	hsa-miR-5693	+	-	enhance	
BID	A/G	rs8190362	hsa-miR-3677-5p	+	-	enhance	
BID	-/CTAT	rs144073937	hsa-miR-4477a	+	-	decrease	
BID	-/CTAT	rs144073937	hsa-miR-4662a-3p	+	-	enhance	
BID	-/CTAT	rs144073937	hsa-miR-659-3p	+	-	decrease	
BID	A/G	rs8190360	hsa-miR-4479	+	-	create	
BID	A/G	rs8190360	hsa-miR-4520a-5p	+	-	break	
BID	A/G	rs8190360	hsa-miR-4520b-5p	+	-	break	
BID	T/C	rs140522332	hsa-miR-4489	+	-	create	
BID	T/C	rs140522332	hsa-miR-4721	+	-	break	
BID	T/G	rs184390244	hsa-miR-4489	+	-	decrease	
BID	T/G	rs184390244	hsa-miR-4764-3p	+	-	break	
BID	T/G	rs184390244	hsa-miR-556-3p	+	-	enhance	
BID	T/G	rs184390244	hsa-miR-644b-5p	+	-	break	
BID	-/AA	rs25591	hsa-miR-4502	+	-	decrease	
BID	-/AA	rs25591	hsa-miR-888-5p	+	-	break	
BID	-/A	rs77339790	hsa-miR-4659a-5p	+	-	decrease	

Table 11.*(Continued)*

D: The derived allele disrupts a protected miRNA region **C: The derived allele creates a new miRNA region** **Break: The derived allele breaks a new miRNA- mRNA binding site** **Create: The derived allele breaks a conserved miRNA- mRNA binding site and creates a new miRNA- mRNA binding site** **Decrease: The derived allele decreases a new miRNA- mRNA binding site** **Enhance: The derived allele enhances a new miRNA- mRNA binding site**

Table 12. SNPs and miRNAs in *MAPK10* gene predicted by miRNA target prediction databases.

Gene	Variation	dbSNP ID	miRNA	MirSNP	PolymiRTS	MirSNP Effect	PolymiRTS Effect
MAPK10	C/A	rs113524529	hsa-miR-6841-5p	-	+		C
MAPK10	T/C	rs193043601	hsa-let-7i-3p	+	+	create	
MAPK10	T/C	rs193043601	hsa-let-7i-3p	+	+		C
MAPK10	T/C	rs193043601	hsa-let-7i-3p	+	+	create	
MAPK10	T/C	rs193043601	hsa-let-7i-3p	+	+		C
MAPK10	G/A	rs142651370	hsa-miR-613	+	-	decrease	
MAPK10	G/A	rs142651370	hsa-miR-4677-5p	+	-	enhance	
MAPK10	G/A	rs142651370	hsa-miR-4482-3p	+	-	break	
MAPK10	G/A G/G	rs142651370	hsa-miR-22-5p	+	+	break	
MAPK10	G/A G/G	rs142651370	hsa-miR-22-5p	+	+		D
MAPK10	G/A G/G	rs142651370	hsa-miR-4659a-3p	+	+	break	
MAPK10	G/A G/G	rs142651370	hsa-miR-4659a-3p	+	+		D
MAPK10	G/A G/G	rs142651370	hsa-miR-4659b-3p	+	+	break	
MAPK10	G/A G/G	rs142651370	hsa-miR-4659b-3p	+	+		D
MAPK10	C/A	rs183690138	hsa-miR-4482-3p	+	-	decrease	
MAPK10	C/A	rs183690138	hsa-miR-4659b-3p	+	-	decrease	
MAPK10	C/A	rs183690138	hsa-miR-4659a-3p	+	-	decrease	
MAPK10	A/C	rs183690138	hsa-miR-494-3p	-	+		C
MAPK10	A/C	rs183690138	hsa-miR-510-3p	-	+		C
MAPK10	G/A	rs1202	hsa-miR-5197-5p	+	-	decrease	
MAPK10	G/A G/G	rs1202	hsa-miR-3622b-5p	+	+	break	
MAPK10	G/A G/G	rs1202	hsa-miR-3622b-5p	+	+		D
MAPK10	G/A G/G	rs1202	hsa-miR-4720-5p	+	+	break	
MAPK10	G/A G/G	rs1202	hsa-miR-4720-5p	+	+		D
MAPK10	G/A G/G	rs1202	hsa-miR-4799-3p	+	+	break	
MAPK10	G/A G/G	rs1202	hsa-miR-4799-3p	+	+		D
MAPK10	G/A G/G	rs1202	hsa-miR-5588-5p	+	+	break	
MAPK10	G/A G/G	rs1202	hsa-miR-5588-5p	+	+		D
MAPK10	G/A	rs1202	hsa-miR-4774-5p	+	+	create	
MAPK10	G/A	rs1202	hsa-miR-4774-5p	+	+		C
MAPK10	G/A	rs1202	hsa-miR-652-3p	-	+		C
MAPK10	G/A	rs1202	hsa-miR-8074	-	+		C
MAPK10	G/A	rs146020875	hsa-miR-5092	-	+		C
MAPK10	T/C	rs138228504	hsa-miR-520g	+	-	break	
MAPK10	T/C	rs138228504	hsa-miR-4679	+	-	enhance	

Table 12. *(Continued)*

MAPK10	T/C	rs138228504	hsa-miR-3134	+	-	decrease	
MAPK10	T/C C/T	rs138228504	hsa-miR-3609	+	+	break	
MAPK10	T/C C/T	rs138228504	hsa-miR-3609	+	+		C
MAPK10	C/T	rs138228504	hsa-miR-3973	-	+		C
MAPK10	C/T	rs138228504	hsa-miR-520g-3p	-	+		C
MAPK10	T/C C/T	rs138228504	hsa-miR-520h	+	+	break	
MAPK10	T/C C/T	rs138228504	hsa-miR-520h	+	+		C
MAPK10	T/C C/T	rs138228504	hsa-miR-548ah-5p	+	+	break	
MAPK10	T/C C/T	rs138228504	hsa-miR-548ah-5p	+	+		C
MAPK10	T/C C/T	rs141818233	hsa-miR-3646	+	+	break	
MAPK10	T/C C/T	rs141818233	hsa-miR-3646	+	+		C
MAPK10	T/G	rs113910535	hsa-miR-519e-3p	+	-	enhance	
MAPK10	T/G	rs113910535	hsa-miR-515-3p	+	-	enhance	
MAPK10	T/G	rs113910535	hsa-miR-3681-3p	+	-	decrease	
MAPK10	T/G	rs113910535	hsa-miR-33b-3p	+	-	decrease	
MAPK10	T/G	rs113910535	hsa-miR-27a-3p	+	-	decrease	
MAPK10	G/G	rs113910535	hsa-miR-6778-3p	-	+		D
MAPK10	G/G	rs113910535	hsa-miR-6791-3p	-	+		D
MAPK10	G/G	rs113910535	hsa-miR-6829-3p	-	+		D
MAPK10	G/G	rs113910535	hsa-miR-6836-3p	-	+		D
MAPK10	G/A	rs17011312	hsa-miR-519e-3p	+	-	enhance	
MAPK10	G/A	rs17011312	hsa-miR-518e-3p	+	-	break	
MAPK10	G/A	rs17011312	hsa-miR-515-3p	+	-	decrease	
MAPK10	G/A	rs17011312	hsa-miR-372	+	-	create	
MAPK10	G/A	rs17011312	hsa-miR-3681-3p	+	-	enhance	
MAPK10	G/A	rs17011312	hsa-miR-33b-3p	+	-	enhance	
MAPK10	G/A	rs17011312	hsa-miR-27b-3p	+	-	enhance	
MAPK10	G/A	rs17011312	hsa-miR-27a-3p	+	-	enhance	
MAPK10	G/A A/A	rs17011312	hsa-miR-302a-3p	+	+	create	
MAPK10	G/A A/A	rs17011312	hsa-miR-302a-3p	+	+		D
MAPK10	G/A A/A	rs17011312	hsa-miR-302b-3p	+	+	create	
MAPK10	G/A A/A	rs17011312	hsa-miR-302b-3p	+	+		D
MAPK10	G/A A/A	rs17011312	hsa-miR-302c-3p	+	+	create	
MAPK10	G/A A/A	rs17011312	hsa-miR-302c-3p	+	+		D
MAPK10	G/A A/A	rs17011312	hsa-miR-302d-3p	+	+	create	
MAPK10	G/A A/A	rs17011312	hsa-miR-302d-3p	+	+		D
MAPK10	G/A A/A	rs17011312	hsa-miR-302e	+	+	create	
MAPK10	G/A A/A	rs17011312	hsa-miR-302e	+	+		D
MAPK10	G/A A/A	rs17011312	hsa-miR-372-3p	+	+	create	
MAPK10	G/A A/A	rs17011312	hsa-miR-372-3p	+	+		D
MAPK10	A/A	rs17011312	hsa-miR-373-3p	-	+		D
MAPK10	A/A	rs17011312	hsa-miR-3934-3p	-	+		D

Table 12. *(Continued)*

MAPK10	A/A	rs17011312	hsa-miR-519a-3p	-	+		D
MAPK10	A/A	rs17011312	hsa-miR-519b-3p	-	+		D
MAPK10	A/A	rs17011312	hsa-miR-519c-3p	-	+		D
MAPK10	G/A A/A	rs17011312	hsa-miR-520a-3p	+	+	create	
MAPK10	G/A A/A	rs17011312	hsa-miR-520a-3p	+	+		D
MAPK10	G/A A/A	rs17011312	hsa-miR-520b	+	+	create	
MAPK10	G/A A/A	rs17011312	hsa-miR-520b	+	+		D
MAPK10	G/A A/A	rs17011312	hsa-miR-520c-3p	+	+	create	
MAPK10	G/A A/A	rs17011312	hsa-miR-520c-3p	+	+		D
MAPK10	G/A A/A	rs17011312	hsa-miR-520d-3p	+	+	create	
MAPK10	G/A A/A	rs17011312	hsa-miR-520d-3p	+	+		D
MAPK10	G/A A/A	rs17011312	hsa-miR-520e	+	+	create	
MAPK10	G/A A/A	rs17011312	hsa-miR-520e	+	+		D
MAPK10	A/G	rs17011312	hsa-miR-518e-3p	-	+		C
MAPK10	T/C C/C	rs188211415	hsa-miR-544b	+	+	create	
MAPK10	T/C C/C	rs188211415	hsa-miR-544b	+	+		D
MAPK10	T/C C/C	rs188211415	hsa-miR-1200	+	+	create	
MAPK10	T/C C/C	rs188211415	hsa-miR-1200	+	+		D
MAPK10	T/C C/C	rs188211415	hsa-miR-3915	+	+	create	
MAPK10	T/C C/C	rs188211415	hsa-miR-3915	+	+		D
MAPK10	C/C	rs188211415	hsa-miR-3928-3p	-	+		D
MAPK10	T/C	rs188211415	hsa-miR-5584-5p	+	-	enhance	
MAPK10	T/C	rs188211415	hsa-miR-5702	+	-	decrease	
MAPK10	C/C	rs188211415	hsa-miR-4324	-	+		D
MAPK10	T/C C/T	rs188211415	hsa-miR-4521	+	+	break	
MAPK10	T/C C/T	rs188211415	hsa-miR-4521	+	+		C
MAPK10	C/T	rs188211415	hsa-miR-651-3p	-	+		C
MAPK10	G/A	rs139770617	hsa-miR-607	+	-	enhance	
MAPK10	G/A	rs139770617	hsa-miR-3200-5p	+	-	create	
MAPK10	G/A G/G	rs139770617	hsa-miR-4324	+	+	break	
MAPK10	G/A G/G	rs139770617	hsa-miR-4324	+	+		D
MAPK10	G/G	rs139770617	hsa-miR-490-3p	-	+		D
MAPK10	G/G	rs139770617	hsa-miR-649	-	+		D
MAPK10	G/A	rs139770617	hsa-miR-3200-5p	-	+		C
MAPK10	G/A	rs139770617	hsa-miR-4251	-	+		C
MAPK10	G/A	rs139770617	hsa-miR-4329	-	+		C
MAPK10	G/A	rs139770617	hsa-miR-6761-5p	-	+		C
MAPK10	T/A	rs7660160	hsa-miR-4795-3p	+	-	decrease	
MAPK10	A/T	rs7660160	hsa-miR-126-5p	-	+		C
MAPK10	C/A	rs17011314	hsa-miR-767-3p	+	-	enhance	
MAPK10	C/A	rs17011314	hsa-miR-587	+	-	create	

Table 12. *(Continued)*

MAPK10	C/A	rs17011314	hsa-miR-584-3p	+	-	decrease	
MAPK10	C/A	rs17011314	hsa-miR-525-5p	+	-	break	
MAPK10	C/A	rs17011314	hsa-miR-520a-5p	+	-	break	
MAPK10	C/A	rs17011314	hsa-miR-3180-5p	+	-	break	
MAPK10	C/A	rs17011314	hsa-miR-3976	+	+	creat	
MAPK10	C/A	rs17011314	hsa-miR-3976	+	+		C
MAPK10	C/A	rs17011314	hsa-miR-6758-5p	-	+		C
MAPK10	C/A	rs17011314	hsa-miR-6856-5p	-	+		C
MAPK10	G/G	rs114030030	hsa-miR-7975	-	+		D
MAPK10	G/A	rs114030030	hsa-miR-593-3p	-	+		C
MAPK10	G/A	rs114030030	hsa-miR-6818-3p	-	+		C
MAPK10	G/A	rs114030030	hsa-miR-6895-3p	-	+		C
MAPK10	T/C	rs200043113	hsa-miR-595	-	+		C
MAPK10	T/C	rs200043113	hsa-miR-597-5p	-	+		C
MAPK10	G/C	rs145685583	hsa-miR-4307	+	-	decrease	
MAPK10	G/C	rs145685583	hsa-miR-3679-3p	+	-	decrease	
MAPK10	G/C	rs145685583	hsa-miR-19a-5p	-	+		C
MAPK10	G/C	rs145685583	hsa-miR-19b-1-5p	-	+		C
MAPK10	G/C	rs145685583	hsa-miR-19b-2-5p	-	+		C
MAPK10	G/C	rs145685583	hsa-miR-2052	-	+		C
MAPK10	T/C	rs184732983	hsa-miR-582-5p	+	-	decrease	
MAPK10	T/C	rs184732983	hsa-miR-495	+	-	break	
MAPK10	T/T	rs184732983	hsa-miR-4275	-	+		D
MAPK10	T/T	rs184732983	hsa-miR-495-3p	-	+		D
MAPK10	T/C T/T	rs184732983	hsa-miR-5688	+	+	break	
MAPK10	T/C T/T	rs184732983	hsa-miR-5688	+	+		D
MAPK10	T/T	rs184732983	hsa-miR-7-1-3p	-	+		D
MAPK10	T/T	rs184732983	hsa-miR-7-2-3p	-	+		D
MAPK10	G/A	rs3733367	hsa-miR-203	+	-	enhance	
MAPK10	G/A G/G	rs3733367	hsa-miR-3619-3p	+	+	Break	
MAPK10	G/A G/G	rs3733367	hsa-miR-3619-3p	+	+		D
MAPK10	G/A G/G	rs3733367	hsa-miR-4776-5p	+	+	break	
MAPK10	G/A G/G	rs3733367	hsa-miR-4776-5p	+	+		D
MAPK10	G/A G/G	rs3733367	hsa-miR-591	+	+	break	
MAPK10	G/A G/G	rs3733367	hsa-miR-591	+	+		D
MAPK10	G/A	rs3733367	hsa-miR-299-3p	-	+		C
MAPK10	G/A	rs3733367	hsa-miR-4491	-	+		C
MAPK10	G/A	rs3733367	hsa-miR-4657	-	+		C
MAPK10	G/A	rs3733367	hsa-miR-6503-3p	-	+		C
MAPK10	T/C	rs188386801	hsa-miR-4732-5p	+	-	create	
MAPK10	T/C	rs188386801	hsa-miR-1233	+	-	create	
MAPK10	C/C	rs188386801	hsa-miR-1233-3p	-	+		D
MAPK10	C/C	rs188386801	hsa-miR-3529-5p	-	+		D

Table 12. *(Continued)*

MAPK10	C/C	rs188386801	hsa-miR-379-5p	-	+		D
MAPK10	T/C C/C	rs188386801	hsa-miR-4451	+	+	create	
MAPK10	T/C C/C	rs188386801	hsa-miR-4451	+	+		D
MAPK10	C/C	rs188386801	hsa-miR-4650-3p	-	+		D
MAPK10	C/C	rs188386801	hsa-miR-4732-5p	-	+		D
MAPK10	G/A	rs958	hsa-miR-1233	+	-	break	
MAPK10	G/G	rs958	hsa-miR-1233-3p	-	+		D
MAPK10	G/A	rs958	hsa-miR-125a	+	-	break	
MAPK10	G/A	rs958	hsa-miR-125b	+	-	break	
MAPK10	G/G	rs958	hsa-miR-125a-5p	-	+		D
MAPK10	G/G	rs958	hsa-miR-125b-5p	-	+		D
MAPK10	G/A G/G	rs958	hsa-miR-4319	+	+	break	
MAPK10	G/A G/G	rs958	hsa-miR-4319	+	+		D
MAPK10	G/A G/G	rs958	hsa-miR-544b	+	+	break	
MAPK10	G/A G/G	rs958	hsa-miR-544b	+	+		D
MAPK10	G/G	rs958	hsa-miR-6779-3p	-	+		D
MAPK10	G/G	rs958	hsa-miR-7976	-	+		D
MAPK10	G/A	rs958	hsa-miR-1224-5p	-	+		C
MAPK10	G/A	rs958	hsa-miR-3915	-	+		C
MAPK10	G/A	rs958	hsa-miR-4689	-	+		C
MAPK10	G/A	rs958	hsa-miR-6858-5p	-	+		C
MAPK10	T/C	rs191358395	hsa-miR-4643	+	-	decrease	
MAPK10	C/C	rs191358395	hsa-miR-4742-3p	-	+		D
MAPK10	A/G	rs3527	hsa-miR-708-3p	+	-	enhance	
MAPK10	A/G	rs3527	hsa-miR-4670-3p	+	-	enhance	
MAPK10	A/G	rs3527	hsa-miR-4643	-	+		C
MAPK10	A/G	rs3527	hsa-miR-466	+	+	create	
MAPK10	A/G	rs3527	hsa-miR-466	+	+		C
MAPK10	A/G	rs3527	hsa-miR-4789-3p	-	+		C
MAPK10	A/A	rs183689080	hsa-miR-6884-3p	-	+		D
MAPK10	T/C	rs146719773	hsa-miR-1182	+	-	decrease	
MAPK10	T/C	rs146719773	hsa-miR-4697-3p	+	-	break	
MAPK10	T/C	rs146719773	hsa-miR-5006-5p	+	-	enhance	
MAPK10	A/T	rs2575674	hsa-miR-1237	+	-	break	
MAPK10	G/A	rs181956061	hsa-miR-1245b-3p	+	-	create	
MAPK10	G/A	rs181956061	hsa-miR-616-3p	+	-	enhance	
MAPK10	G/A	rs181956061	hsa-miR-5683	+	-	create	
MAPK10	T/A	rs192987418	hsa-miR-1271-3p	+	-	enhance	
MAPK10	T/A	rs192987418	hsa-miR-548a-3p	+	-	break	
MAPK10	T/A	rs192987418	hsa-miR-548ar-3p	+	-	break	
MAPK10	T/A	rs192987418	hsa-miR-548e	+	-	break	
MAPK10	T/A	rs192987418	hsa-miR-548f	+	-	break	

Table 12. *(Continued)*

MAPK10	T/A	rs192987418	hsa-miR-548g-3p	+	-	break	
MAPK10	T/A	rs192987418	hsa-miR-550a-3-5p	+	-	decrease	
MAPK10	T/A	rs192987418	hsa-miR-550a-5p	+	-	decrease	
MAPK10	T/A	rs192987418	hsa-miR-550b-2-5p	+	-	decrease	
MAPK10	T/C	rs143420758	hsa-miR-1289	+	-	break	
MAPK10	T/C	rs143420758	hsa-miR-1915-3p	+	-	create	
MAPK10	T/C	rs143420758	hsa-miR-4433-3p	+	-	break	
MAPK10	T/C	rs143420758	hsa-miR-4772-3p	+	-	decrease	
MAPK10	T/C	rs143420758	hsa-miR-615-5p	+	-	create	
MAPK10	T/C	rs143420758	hsa-miR-665	+	-	break	
MAPK10	T/C	rs113486237	hsa-miR-1304-3p	+	-	break	
MAPK10	T/C	rs113486237	hsa-miR-639	+	-	create	
MAPK10	T/C	rs113486237	hsa-miR-720	+	-	create	
MAPK10	G/A	rs189486762	hsa-miR-1915-3p	+	-	decrease	
MAPK10	G/A	rs189486762	hsa-miR-370	+	-	create	
MAPK10	G/A	rs189486762	hsa-miR-4649-3p	+	-	create	
MAPK10	G/A	rs189486762	hsa-miR-615-5p	+	-	decrease	
MAPK10	G/A	rs189486762	hsa-miR-93-3p	+	-	create	
MAPK10	-/CA	rs72168477	hsa-miR-20a-3p	+	-	enhance	
MAPK10	-/CA	rs72168477	hsa-miR-3650	+	-	create	
MAPK10	-/CA	rs72168477	hsa-miR-4288	+	-	enhance	
MAPK10	-/CA	rs72168477	hsa-miR-5010-3p	+	-	break	
MAPK10	-/CA	rs72168477	hsa-miR-632	+	-	enhance	
MAPK10	-/CA	rs72168477	hsa-miR-632	+	-	decrease	
MAPK10	T/G	rs150641306	hsa-miR-302b-3p	+	-	decrease	
MAPK10	T/G	rs150641306	hsa-miR-372	+	-	decrease	
MAPK10	T/G	rs150641306	hsa-miR-5696	+	-	create	
MAPK10	G/A	rs147506110	hsa-miR-3945	+	-	break	
MAPK10	G/A	rs147506110	hsa-miR-4461	+	-	decrease	
MAPK10	G/A	rs147506110	hsa-miR-4506	+	-	create	
MAPK10	G/A	rs147506110	hsa-miR-539-5p	+	-	enhance	
MAPK10	-/C	rs35473638	hsa-miR-4271	+	-	create	
MAPK10	-/C	rs35473638	hsa-miR-4725-3p	+	-	create	
MAPK10	-/AC	rs72228542	hsa-miR-4288	+	-	break	
MAPK10	-/AC	rs72228542	hsa-miR-4520a-5p	+	-	break	
MAPK10	-/AC	rs72228542	hsa-miR-4520b-5p	+	-	break	
MAPK10	-/AC	rs72228542	hsa-miR-632	+	-	break	
MAPK10	-/CA	rs112287084	hsa-miR-4288	+	-	decrease	
MAPK10	-/CA	rs112287084	hsa-miR-4288	+	-	enhance	
MAPK10	-/CA	rs112287084	hsa-miR-632	+	-	decrease	
MAPK10	-/CA	rs112287084	hsa-miR-632	+	-	enhance	
MAPK10	G/C	rs140220627	hsa-miR-4506	+	-	enhance	

Table 12. *(Continued)*

MAPK10	T/C	rs1201	hsa-miR-4709-3p	+	-	enhance	
MAPK10	C/G	rs2589515	hsa-miR-4758-3p	+	-	break	
MAPK10	C/G	rs2589515	hsa-miR-548a-3p	+	-	enhance	
MAPK10	C/G	rs2589515	hsa-miR-548ar-3p	+	-	enhance	
MAPK10	C/G	rs2589515	hsa-miR-548e	+	-	enhance	
MAPK10	C/G	rs2589515	hsa-miR-548g-3p	+	-	enhance	
MAPK10	-/CA	rs149235688	hsa-miR-632	+	-	enhance	
MAPK10	-/CA	rs149235688	hsa-miR-632	+	-	decrease	
MAPK10	-/CA	rs149235688	hsa-miR-4288	+	-	enhance	
MAPK10	-/CA	rs149235688	hsa-miR-4288	+	-	decrease	
D: The derived allele disrupts a protected miRNA region **C: The derived allele creates a new miRNA region** **Break: The derived allele breaks a new miRNA- mRNA binding site** **Create: The derived allele breaks a conserved miRNA- mRNA binding site and creates a new miRNA- mRNA binding site** **Decrease: The derived allele decreases a new miRNA- mRNA binding site** **Enhance: The derived allele enhances a new miRNA- mRNA binding site**							

IV. DISCUSSION

SNPs are important types of polymorphisms in the early diagnosis of many hereditary or multifactorial diseases or in determining susceptibility to diseases. Therefore, it is of great importance to predict harmful SNPs by computational methods such as *in silico* methods. In this study, the harmful SNPs and mirSNPs of the *BID, MAPK10,* and *AGER* genes, which were previously determined to be related to AD, were identified by *in silico* analysis.

A. Discussion of Gene-Gene and Protein-Protein Interactions

Researching protein-protein and gene-gene interactions has become the right approach to investigating the etiology and neuropathology of complex diseases such as AD [47, 48]. With these studies, the complexity of many diseases, including AD, has started to be solved [47, 48]. The treatment is one step closer by identifying the genes and proteins associated with the disease or the molecules critical to treating the disease. With these studies, the researchers identified the candidate genes and signal pathways involved in AD pathogenesis [47, 48].

In this study, the most closely related genes and proteins were identified using GeneMANIA (Figure 2), which provides gene-gene interactions, and STRING tool (Figure 3) which offers protein-protein interactions to determine the possible tasks of genes. In addition, the pathways in which these genes and proteins are found have been determined.

B. Discussion of the Results of Functional Analysis of SNPs

Based on the results obtained from studies, it has been found that programs with various algorithms are systematically used to uncover harmful/disease-related SNPs. Therefore, the use of evolutionary knowledge in the creation of such programs is of great importance [49]. SIFT and PolyPhen-2 programs have been shown to be good results and used to identify the most harmful SNPs [50, 51]. SIFT, PolyPhen-2, PROVEAN, SNPs&GO, Mutation Assessor, SNAP2, PHD-SNP, PANTHER, and META-SNP software tools were used to identify the possible effects of SNPs in this study. The results showed that 7 SNPs in the *BID* gene, 27 SNPs in the *MAPK10* gene, and 3 SNPs in the *AGER* gene are predicted as high-risk SNPs by *in silico* tools (Table 1 and Table 2). There is no experimental studies have been found in the literature on those SNPs that predicted as deleterious in this study. Only one study conducted about the *AGER* gene has shown that the G82S variant (rs2070600) is associated with AD [52]. In this study, it was found that this SNP was associated with harmful/ disease-related in 4 out of 10 results obtained from software tools, and neutral in 6 of them.

C. Discussing the Effects of SNPs on Protein Stabilization

I- Mutant and MUpro software tools were used to detect stability changes made by all identified harmful SNPs in protein structure. All SNPs analyzed in the *BID, MAPK10,* and *AGER* genes have been found to reduce

protein stabilization in both programs (Table 3). When protein stability is disrupted, the protein cannot be folded properly. Therefore, a protein must have a stable conformation to fold and perform its function correctly. Due to SNPs in protein structure, ligand binding of proteins, allosteric matches, general folding stability and post-translation modification of natural protein may be impaired [47, 52-54].

D. Discussing the Possible Effects of Amino Acid Substitutions

It is known that the position of amino acids influenced by the SNP is very important to understand the mechanisms of polymorphisms in diseases [55, 56]. The part where the amino acid change occurs can be a very important region for protein. In this case, it can cause the deterioration of hydrogen bonds caused by the change in that region, disruption of its conformation, differentiation of the interaction network, deterioration of the salt bridges created, and serious differences in the protein such as conformation changes and energy changes [56, 57]. Such changes can also cause alteration of protein stability, disruption of folding kinetics, and protein aggregations [57]. Project HOPE software was used to obtain features of wild and variant type residues and three-dimensional structure of the protein (Table 4-9).

The size characteristics of proteins are generally related with larger domains formed by many of their residences [58]. This size difference can cause different results according to the location of the variant amino acid. If the wild type amino acid is larger than the mutant type amino acid such as L19P, L151P, L105P, and F171V variants in the BID protein and Y268C, L95P, R188M, W390R, and R63C variants in the MAPK10 protein, it is thought to cause loss of interactions with other molecules (Table 4-5) [42]. In addition, this may cause an empty area in the core of the protein in positions W272R, M339I, C251Y, W247R, L190S and V53G in the MAPK10 protein (Table 5) [42]. In cases where the mutant type amino acid is larger than the wild type amino acid such as N181K, G8V, and G8D variants in the BID protein, G237E variant in the MAPK10 protein, C144W and C38W variants in the AGER protein, the variant type amino acid may cause bumps (Table 4-6) [42]. However, if wild-type amino acid is located in the core of the protein, the variant amino acid will not fit in that region such as A91V, D189Y, L153F, L190F, A231T, G250R, V244L, D141H, A353D, V145L, C250W, V244M, and N122Y variants in MAPK10 protein (Table 5) [42].

Hydrophobic interactions are not real bonds, they are also defined as a measure of how much the side chains of amino acid push water. Hydrophobicity is estimated to be the most important feature in the provision of protein's 3D structure [59]. In protein structure, hydrophobic amino acids are usually located in the inland regions, while hydrophilic regions are located on the outside, contributing to the stable structure of the protein [60]. Molecules with similar hydropathy affinity pull each other, while molecules with different hydropathies push each other so much. The difference in hydrophobicity caused by harmful SNPs between amino acid variants can affect protein structure and function, causing deterioration of protein stability [59-61]. In particular, the disease-causing SNPs have been shown to occur in hydrophobic areas [62]. Wild-type amino acids may be more hydrophobic than variant-type amino acids. In this case, different effects may occur depending on the location of the wild-type amino acid. For example, due to the amino acid substitution in R188M and W390R found in MAPK10 protein and C301S found in the AGER protein, there may be a loss of hydrophobic interactions (Table 5-6). It is also thought that hydrophobic interaction losses may occur due to the W272R, A231T, C251Y, A353D, L190S, N122Y, and V53G variations in the nucleus of the MAPK10 protein (Table 5) [42]. In the D189Y variation of MAPK10 protein, it is thought that as a result of the special functions of wild-type amino acid in the core of the protein, hydrogen bonds may be loss and folding problems may occur in the protein (Table 5) [42].

The charge of amino acids is determined depending on the pH value. Of the 64 existing codons, only 16 encode charged amino acids. Therefore, the effect of electrical charge on the positioning of amino acids is thought to be much less than hydrophobic forces [42]. The charge difference caused by harmful SNPs among amino acid variants can affect its structure and function by disrupting protein stability [59-61]. Variation creates a large charge difference between wild and variant-type amino acids in the N181K variation in the BID protein and the W390R variation in the MAPK10 protein (Table 4 and Table 5). The charge of wild-type amino acid was neutral, while mutant-type has a positive charge so estimated that this may cause the pushing of ligands or other residues with the same load as the mutant residues [42]. In some cases, as in the W272R, G250R, A353D, and W247R variations formed in the MAPK10 protein, it may cause protein folding problems depending on the location of the wild type amino acid (Table 5) [42]. Another case is that the wild-type amino acid in the G8D in the BID protein and G237E in the MAPK10 protein has neutral electrical properties, while the mutant-type amino acid has a negative electrical charge (Table 4 and Table 5). This can lead to the pushing of ligands or other residues with the same load [42]. R188M and R63C variations in the MAPK10 protein can cause loss of charge so cause interaction losses with other molecules (Table 5) [42].

In addition, the degradation of salt bridges or hydrogen bonds in the biochemical structure of amino acids can completely change both the structure and the function of the protein [60, 63]. In particular, disease-causing

SNPs have been shown to occur in hydrogen-bonding amino acids [62]. It is estimated that most polymorphisms in the *MAPK10* gene can disrupt such interactions (Table 10).

E. Discussion of SNP and miRNA Analyses

miRNAs play a role in many biological mechanisms such as disease and infection formation. In addition, they are also involved in many cellular events, such as cell proliferation and cell differentiation. miRSNPs can affect pri-miRNA/pre-miRNA formation and processing. miRSNPs effect the interaction between miRNA and mRNA. Also, they can affect the transcription of the target gene. As a result of many studies, more than 240 rare mutations and SNPs have been identified in pri-miRNA, pre-miRNA, and mature miRNA sequences [64-70]. Genomic and epigenetic as well as SNPs changes are thought to have influenced the reorganization of the pathways of miRNAs [71]. It has been shown by various studies that variations in miRNA sequences or their sequences in target regions cause miRNA to fail to function [72-75]. Previous studies showed that SNPs that disrupt miRNA synthesis are more than SNPs that increase miRNA synthesis [76]. Variations in miRNA binding regions have been determined to be associated with loss of miRNA function [77-82]. Additionally, miRNAs are defined in AD as important elements for regulating lost cognitive functions and memory processes [83].

The effects of SNPs on the miRNA-mRNA sites were obtained from MirSNP and PolyMiRTS software tools and the results were given in the Table 11-12. An in vitro miRNA study conducted in relation to the *BID* gene has shown that decreased mir-124-3p miRNA increases the expression of the *BID* gene. In AD, mir-124-3p miRNA has been shown to decrease and *BID* activity has increased [84]. In this study, it is estimated that rs181869684 polymorphism increases the functional effect of the miRNA- mRNA binding pair for mir-124-3p in the analysis carried out in the MirSNP software (Table 11). An in vitro and in vivo miRNA study associated with the *MAPK10* gene has shown that mir-27a-3p miRNA regulates the expression of the *MAPK10* gene [85]. In this study, it is estimated that rs17011312 polymorphism increases the functional effect of the miRNA- mRNA binding pair for mir-27a-3p, while rs113910535 polymorphism reduces the functional effect of the miRNA- mRNA binding pair (Table 12). Another miRNA study of in vitro and in vivo in relation to the *MAPK10* gene has shown that the reduction of miR-335-5p miRNA increases the expression of the *MAPK10* gene inverse proportion [86]. The programs used in this study did not show the SNP interaction associated with this miRNA.

V. CONCLUSION

The possible effects of 186, 204, and 392 missense SNPs within the *BID*, *MAPK10* and *AGER* genes were evaluated using different computational tools and a total of 7, 27, and 3 of them were suggested as high-risk SNPs, respectively, in this study. In addition, the effects of SNPs in these genes on miRNA-mRNA binding sites were investigated. Although predicting the pathogenic effects of SNPs using bioinformatic tools is advantageous in terms of reducing cost and time, experimental studies are required to understand the effects of SNPs on diseases. This *in silico* study could serve as a basis for targeting pathological SNPs in *BID, MAPK10,* and *AGER* genes for genotyping studies.

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