Turkish Journal of Science & Technology
19(1), 213-222, 2024

Research Paper
https://doi.org/10.55525/tjst.1344460

Evaluation of SNP in the *CDH8* and *CDH10* Genes Associated with Autism Using *In-Silico* Tools

Azadeh REZAEIRAD[1], Ömer Faruk KARASAKAL[2*], Tuğba KAMAN[3], Mesut KARAHAN[4]

[1] Institute of Science, Molecular Biology, Üsküdar University, Istanbul, Türkiye
[2,4] Vocational School of Health Services, Medical Laboratory Techniques, Üsküdar University, Istanbul, Türkiye
[3] Department of Medicinal and Aromatic Plants, Vocational School of Health Services, Üsküdar University, İstanbul, Türkiye
[*2] omerfaruk.karasakal@uskudar.edu.tr

(Geliş/Received: 17/08/2023; Kabul/Accepted: 22/03/2024)

Abstract: Autism spectrum disorder (ASD) is defined as a pervasive and multifactorial neurodevelopmental disorder (ND). It is characterized by repetitive behavioral patterns as well as symptoms of social interaction and communication disorder. The cadherin (CDH) superfamily is a large group of synaptic cell adhesion molecules and has been widely related with ND, including autism. The aim of this study is to evaluate the potentially deleterious missense single nucleotide polymorphisms (SNPs) in *CDH8* and *CDH10* genes, which are related with ASD and cause amino acid changes, using internet-based software tools. To identify potentially harmful missense SNPs; all SNPs were screened using SIFT, PolyPhen-2, PROVEAN, SNPs&GO, Meta-SNP, and SNAP2 software tools, and common deleterious ones were filtered out. Its effect on protein stabilization was investigated with I-Mutant 3.0 and MUpro tools. Three-dimensional models of these common damaging amino acid changes were evaluated with the HOPE software. As a result of in silico analysis of 577 missense SNPs in the *CDH8* gene; The rs145143780 (Y572C) polymorphism common damaging SNP has been detected by all software tools. According to the results of the in silico analysis of 526 missense SNPs found in the *CDH10* gene; The rs13174039 (V459G), rs147882578 (N485K), rs201423740 (Y306C), rs201956238 (F317L) and rs373340564 (R128C) common damaging SNPs have been identified in all polymorphisms by all software tools. As a result of this study, it is thought that the data obtained will make important contributions to future relevant experimental studies.

Key words: Single nucleotide polymorphism (SNP), *CDH8*, *CDH10*, *in silico*, autism spectrum disorder (ASD).

In-Silico Araçlar Kullanılarak Otizmle İlişkili *CDH8* ve *CDH10* Genlerindeki SNP'lerin Değerlendirilmesi

Öz: Otizm spektrum bozukluğu (OSB), yaygın ve çok faktörlü bir nörogelişimsel bozukluk (NB) olarak tanımlanır. Tekrarlayan davranış kalıplarının yanı sıra sosyal etkileşim ve iletişim bozukluğu belirtileri ile karakterizedir. Kadherin (CDH) süper ailesi, büyük bir sinaptik hücre adezyon molekülleri grubudur ve otizm de dahil olmak üzere NB ile geniş çapta ilişkilendirilmiştir. Bu çalışmanın amacı, otizm spektrum bozukluğu ile ilişkili ve amino asit değişikliklerine neden olan *CDH8* ve *CDH10* genlerinde potansiyel olarak zararlı olan missense tek nükleotid polimorfizmlerinin internet tabanlı yazılım araçları kullanılarak değerlendirilmesidir. Potansiyel olarak zararlı yanlış anlamlı SNP'leri tanımlamak için; tüm SNP'ler SIFT, PolyPhen-2, PROVEAN, SNPs&GO, Meta-SNP ve SNAP2 yazılım araçları kullanılarak tarandı ve ortak zararlı olanlar filtrelendi. Protein stabilizasyonu üzerindeki etkisi, I-Mutant 3.0 ve MUpro araçlarıyla araştırıldı. Bu ortak zararlı amino asit değişikliklerinin üç boyutlu modelleri HOPE yazılımı ile değerlendirildi. *CDH8* genindeki 577 missense SNP'nin *in silico* analizi sonucunda; SNP'ye zarar veren rs145143780 (Y572C) polimorfizmi tüm yazılım araçları tarafından tespit edilmiştir. *CDH10* geninde bulunan 526 missense SNP'nin *in silico* analiz sonuçlarına göre; rs13174039 (V459G), rs147882578 (N485K), rs201423740 (Y306C), rs201956238 (F317L) ve rs373340564 (R128C) ortak zarar veren SNP'ler, tüm polimorfizmlerde tüm yazılım araçları tarafından tanımlanmıştır. Bu çalışma sonucunda elde edilen verilerin gelecekte ilgili deneysel çalışmalara önemli katkılar sağlayacağı düşünülmektedir.

Anahtar kelimeler: Tek nükleotid polimorfizmi, *CDH8*, *CDH10*, *in silico*, otizm spektrum bozukluğu (OSB).

1. Introduction

Autism spectrum disorder (ASD) is a multifactorial, pervasive neurodevelopmental disorder (ND) characterized by restricted, repetitive behavioral patterns as well as core symptoms of significant impairment in social interaction and communication [1]. ASD has been reported as a psychiatric pathology that affects almost 1% of the world's population, is more common in men (male/female ratio 4.3:1) and is generally common in children under 3 years of age [2]. Common early signs and symptoms in these children include not responding when called by name, no or very limited use of gestures in the communication process, and lack of creative play [3]. ASD is a serious ND with a strong genetic basis. However, the genetic contributions to ASD are extremely

* Corresponding author: omerfaruk.karasakal@uskudar.edu.tr . ORCID Number of authors: [1] 0000-0002-9349-1149, [2] 0000-0001-7803-3249, [3] 0000-0002-5885-0193, [4] 0000-0002-8971-678X

heterogeneous, and many different loci have been reported to underlie the disease to varying degrees in different individuals [4]. Despite the evidence that harmful variants in the same genes play a role in multiple ND, there has been great interest in identifying genes that, when mutated, confer a large degree of ASD-specific risk [5].

The cadherin superfamily is a large group of synaptic cell adhesion molecules and has been widely related to ND, including ASD [6,7]. Cadherin-8, which is encoded by the *CDH8* gene, a gene associated with ASDs, is known as the homophilic adhesion protein. This gene is type II from the cadherin superfamily and encodes integral membrane proteins that mediate calcium-dependent cell-cell adhesion [8]. The *CDH10* gene encodes the type II classical cadherin protein and is located in the cortex, which is thought to be critical in ASD. In addition, it has tasks such as regionalization of the brain, formation of neural circuits, and plasticity [9-11].

Among the variations in the human genome, the most common are SNPs [12]. SNPs are divided into non-synonymous ones located in the coding region of the target gene. Missense SNPs are amino acid changes that cause changes in the function of proteins [13]. These functional changes may have different impacts on the structure and function of the protein. For example, gene regulation can affect its modulation, protein hydrophobicity, charge, stability, interactions, and translation [14]. For these reasons, missense SNPs are associated with various diseases [15]. *In-silico* studies are used to analysis the impacts of variants on the structure and function of proteins [16]. Bioinformatics software tools provide significant advantages to researchers. Especially with *in silico* analysis performed before experimental studies, savings can be made in terms of cost and time. In addition, it provides rapid prediction for many compounds, allows obtaining pioneering data in drug development activities, and missense SNPs with possible harmful effects in genes known to be associated with diseases can be detected, as in our study. Thanks to this detection, instead of analyzing hundreds or thousands of SNPs, those predicted *in silico* can be studied first in research [17-19].

The aim of this study is to evaluate the potentially deleterious missense SNPs in the *CDH8* and *CDH10* genes, which are related with ASD and which cause amino acid changes, using internet-based software tools. In this relationship, it includes the evaluation of the possible consequences of the findings by estimating the possible effects of SNPs on the function, structure, and stabilization of the protein.

2. Materials and Methods

In this study, the possible impacts of missense SNPs in *CDH8* and *CDH10* genes related with ASD on protein structure, stabilization, and function were estimated, and possible high-risk SNPs that are common harms in all software tools were determined. In this context, the steps of the method applied to the selection of the target genes in the study are as follows (Figure 1).

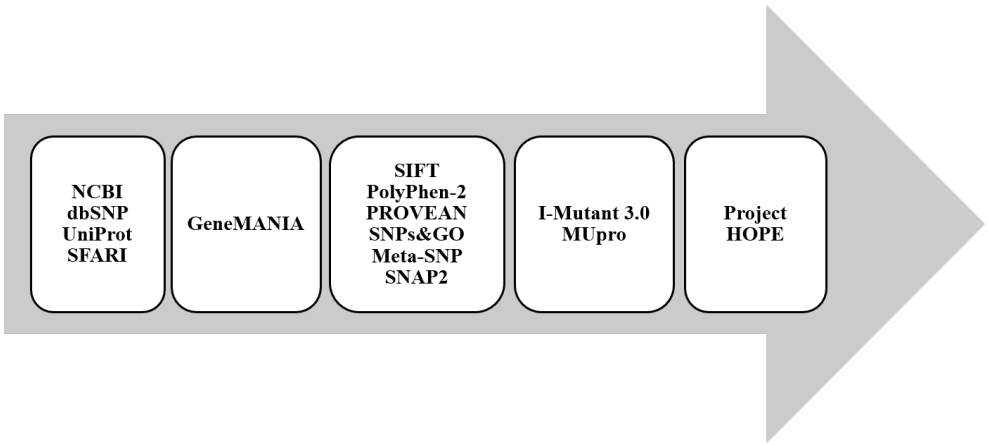


Figure 1. Workflow Diagram

2.1. Data mining

Literature searches and the SFARI database (https://gene.sfari.org) were used to identify the genes to be analyzed in the study. SFARI Gene is an evolving online database designed to help researchers track the ever-expanding genetic risk factors emerging in the literature [20]. SNPs in the *CDH8* and *CDH10* genes were obtained from the NCBI dbSNP database (https://www.ncbi.nlm.nih.gov/snp/). Among these SNPs, missense SNPs were selected.

The protein sequences encoded by the *CDH8* and *CDH10* genes and access codes were obtained from NCBI Gene (https://www.ncbi.nlm.nih.gov/gene/) and UniProt databases. (http://www.uniprot.org/).

2.2. Determination of gene-gene interactions

The relationships between the genes analyzed with bioinformatics tools and gene-gene interactions were determined via GeneMANIA (https://genemania.org). GeneMANIA is a comprehensive software tool that explores publicly available biological data [21,22].

2.3. Bioinformatics evaluation of missense SNPs

In order to estimate the possible effects of missense SNPs in *CDH8* and *CDH10* genes on protein function and structure, SIFT, PolyPhen-2 (Hum-Div, Hum-Var), PROVEAN, SNPs&GO, Meta-SNP, and SNAP2 software tools were used, and common damaging ones were identified in all of them. The variants predicted to be potentially harmful in each of the tools used were selected for further analysis.
SIFT (Sorting Intolerant From Tolerant) (https://sift.bii.a-star.edu.sg/) analyzes the physical properties and sequence homology of amino acids to understand whether an existing amino acid change in protein sequences causes a change in protein function [23]. The PolyPhen-2 (Polymorphism Phenotyping-2) (http://genetics.bwh.harvard.edu/pph2/) software tool is divided into two Hum-Div and Hum-Var. A tool for predicting the possible impact of amino acid changes on the function of human proteins using evolutionary and physical considerations is a software tool [24,25]. PROVEAN (Protein Variation Effect Analyzer) (http://provean.jcvi.org/index.php) is a publicly available tool that determines whether an amino acid change or minor genetic variation has an effect on protein functionality [26,27]. SNPs&GO (http://snps.biofold.org/snps-and-go/snpsand-go.html), is defined as an SVM (Support Vector Machine) based web server used to estimate whether variations in protein structure are associated with the disease [28,29]. Meta-SNP (http://snps.biofold.org/meta-snp/) is a software tool that predicts SNPs are likely to be included in polymorphism or disease classification [30,31]. SNAP2 (https://www.rostlab.org/services/SNAP/) estimates the impact of missense SNPs on protein function. SNAP2 generates predictions based on machine learning methods [32,33].

2.4. Evaluation of the effect of predicted deleterious SNPs on protein stabilization

At this point, SIFT, PolyPhen-2 (Hum-Div, Hum-Var), PROVEAN, SNPs&GO, Meta-SNP, and SNAP2 were used for each amino acid change, and common harms were identified in all of them. After, the effects of these detected variants on protein stabilization were analysis with I-Mutant 3.0 and MUpro. The I-Mutant 3.0 (http://gpcr2.biocomp.unibo.it/cgi/predictors/I-Mutant3.0/I-Mutant3.0.cgi) and MUpro (http://mupro.proteomics.ics.uci.edu/) tools were used to estimate the impacts of predicted potentially deleterious SNPs on protein stabilization. Both of these tools are publicly available tools that estimate the impacts of variants at a single location on the stabilization of proteins [34-36].

2.5. 3D-modeling of predicted harmful variations

Using the Project HOPE software, 3D models of proteins with amino acid changes, which are common in all software tools, were created. Project HOPE (https://www3.cmbi.umcn.nl/hope/) is a software tool that performs calculations on the three-dimensional structure of mutated proteins and provides information about the structural effects of mutation [37,38].

3. Results

The information about the SNPs in the *CDH8* and *CDH10* genes was obtained from the NCBI dbSNP database in December 2021. A total of 142242 SNPs were found in the *CDH8* gene. When the missense SNPs were filtered out among the 142242 SNPs, it was determined that only 577 of them were missense SNPs. A total of 620 different variants of these SNPs were determined. Also, a total of 59691 SNPs were found in the *CDH10* gene. When the SNPs with false meaning among 59691 SNPs were filtered, it was determined that only 526 of them were missense SNPs. A total of 615 different variants of these SNPs were reported.
When the missense SNPs in the *CDH8* gene found to be harmful in all programs were evaluated, the rs145143780 (Y572C) polymorphism was determined to be the most common damaging SNP. In the evaluation of SNPs in the *CDH10* gene that is harmful in all programs, rs13174039 (V459G), rs147882578 (N485K), rs201423740 (Y306C),

rs201956238 (F317L), and rs373340564 (R128C) polymorphisms have all been identified as the most common damaging SNPs.

3.1. Results of SNPs on protein stabilization

One variant of the *CDH8* gene, which was determined to be associated with disease in all software tools, was analyzed with I-Mutant 3.0 and MUpro tools. As a result of the study, it has been determined that one variant has the effect of reducing protein stabilization in both programs (Table 1). In addition, five variants of the *CDH10* gene, which were determined to be related with diseases in all software tools, were analyzed with the I-Mutant 3.0 and MUpro. As a result of the study, it was determined that five variants had a reducing effect on protein stabilization in both programs (Table 1).

Table 1. Results of stabilization of *CDH8* and *CDH10* genes

Gene Name	SNP Number	Amino Acid Change	I-Mutant Result	I-Mutant Reliability Value	DDG (Kcal/mol)	MUpro Result	Mupro DDG value
CDH8	**rs145143780**	Y572C	Decrease	2	0.95	Decrease	-1.0298355
CDH10	**rs13174039**	V459G	Decrease	9	-2.07	Decrease	-1.9914738
	rs147882578	N485K	Decrease	8	-2.29	Decrease	-1.2179979
	rs201423740	Y306C	Decrease	3	0.37	Decrease	-1.1597179
	rs201956238	F317L	Decrease	3	-2.19	Decrease	-1.417702
	rs373340564	R128C	Decrease	2	-0.15	Decrease	-0.18078604

3.2. Results of CDH8 and CDH10 gene-gene interaction

Interactions between genes were determined using the GeneMANIA software tool, and functional and interactional information for two genes was obtained. The interaction of the *CDH8* gene with *CTNND1*, *JUP*, *CTNNA1*, *CDH11*, and PSEN1 genes the maximum has been identified. Also, *CDH10* gene with *CTNND1*, *JUP*, *CTNNA1*, *CTNNB1*, and *CDH1* genes the maximum interaction has been identified. The gene-gene interaction network of the *CDH8* and *CDH10* genes is given in Figure 2.

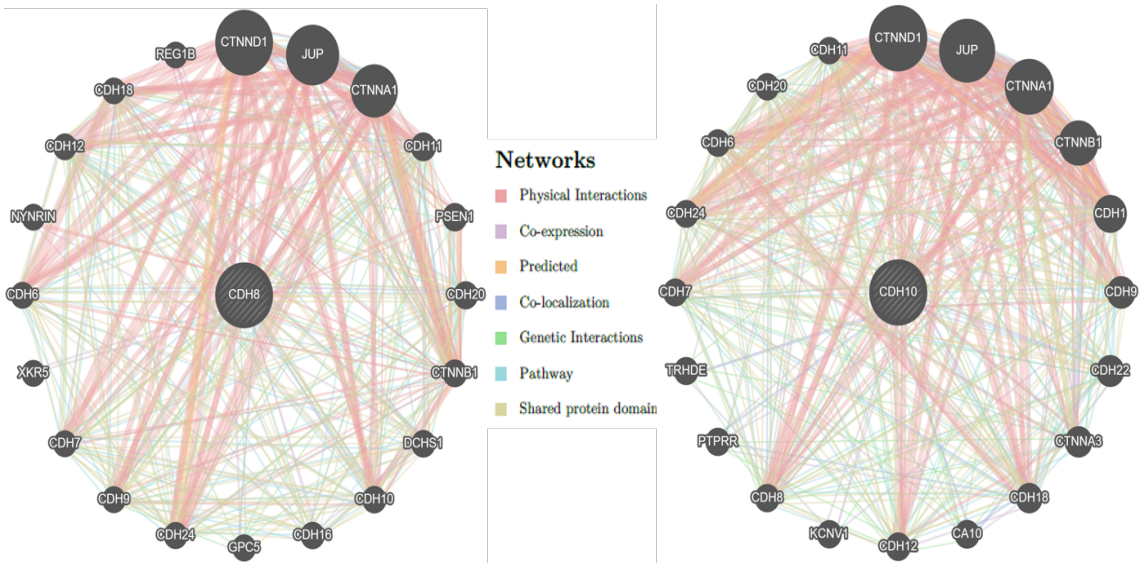


Figure 2. *CDH8* and *CDH10* gene-gene interaction network

3.3. Bioinformatics Analysis Results

Belonging to the *CDH8* and *CDH10* genes associated with autism, gene-gene interaction, prediction of possible damaging effects of SNPs, estimation of the impacts of SNPs on protein stabilization, and a three-dimensional modeling study and its results are presented below. Software tools such as SIFT, PolyPhen-2 (Hum-Div, Hum-Var), PROVEAN, SNPs&GO, SNAP2, and Meta-SNP were used to identify common harms using missense SNPs obtained from the NCBI dbSNP and amino acid change information of each SNP based on variants in the protein sequences (Table 2).

Table 2. Prediction results of *CDH8* and *CDH10* genes SNP in all software tools

Gene Name	SNP ID	Amino Acid Change	SIFT Result	SIFT Score	PolyPhen-2 Result (HumDiv)	PolyPhen-2 Score (HumDiv)	PolyPhen-2 Result (HumVar)	PolyPhen-2 Score (HumVar)	PROVEAN Result	PROVEAN Score	SNPs&GO Result	SNPs&GO Reliability Value	SNAP-2 Result	SNAP-2 Score	SNAP-2 Expected Accuracy	Meta-SNP Result	Meta-SNP Score
CDH8	**rs145143780**	Y572C	Dlt	0.002	PD	1.000	PD	0.997	Dlt	-7.172	Ds	2	Effect	48	71%	Ds	0.721
CDH10	**rs13174039**	V459G	Dlt	0	PD	0.998	PD	0.978	Dlt	-6.651	Ds	2	Effect	78	85%	Ds	0.661
	rs147882578	N485K	Dlt	0.015	PD	0.977	PSD	0.727	Dlt	-4.576	Ds	2	Effect	34	66%	Ds	0.74
	rs201423740	Y306C	Dlt	0.015	PD	1.000	PD	1.000	Dlt	-7.654	Ds	5	Effect	53	75%	Ds	0.823
	rs201956238	F317L	Dlt	0.011	PD	0.995	PSD	0.786	Dlt	-5.105	Ds	5	Effect	54	75%	Ds	0.785
	rs373340564	R128C	Dlt	0.028	PD	1.000	PD	0.999	Dlt	-5.043	Ds	5	Effect	19	59%	Ds	0.751

Dlt: Deleterious, Ds: Disease, PSD: Possibly Damaging, PD: Probably Damaging

3.4. Modeling results for *CDH8* and *CDH10* genes

The mutant residue resulting from the polymorphism often differs from the original wild-type residue. These differences are the charge, hydrophobicity, and size values specific to amino acids. The patterns of amino acid changes resulting from polymorphisms and the differences between residues are presented in Table 3.

Table 3. Wild and mutant-type residue properties of the *CDH8* and *CDH10* determined using Project HOPE

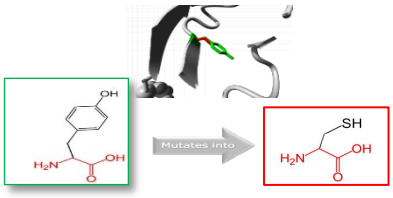
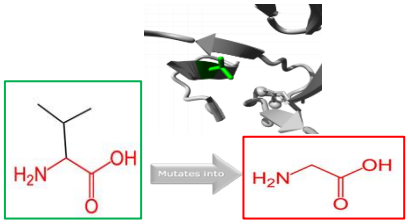
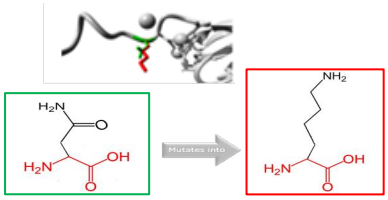
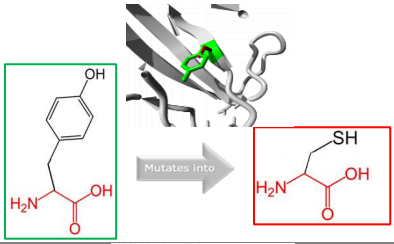
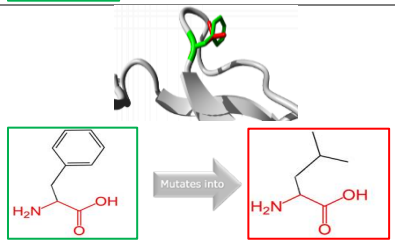
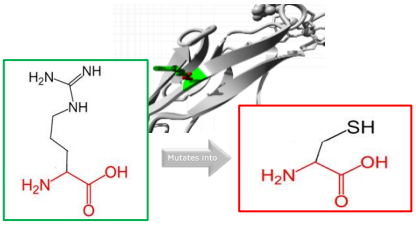
Gene Name	SNP ID	AMINO ACID CHANGE	WILD-TYPE RESIDUES			MUTANT-TYPE RESIDUES		
			Size	Charge	Hydrophobicity	Size	Charge	Hydrophobicity
CDH8	**rs145143780**	Y572C	>	-	<	<	-	>
CDH10	**rs13174039**	V459G	>	-	>	<	-	<
	rs147882578	N485K	<	Neutral	-	>	+charge	-
	rs201423740	Y306C	>	-	<	<	-	>
	rs201956238	F317L	>	-	-	<	-	-
	rs373340564	R128C	>	+charge	<	<	Neutral	>

According to the results, it was determined that the amino acid change created by SNP belonging to the *CDH8* gene, which was predicted to be high risk, was Y572C. Furthermore, amino acid changes created by high-risk SNPs of the *CDH10* gene have been identified as V459G, N485K, Y306C, F317L, and R128C. The 3D model of the protein created using the Project HOPE tool is shown in Table 4.

4. Discussions

In this study, estimation results of the possible impacts of SNPs in *CDH8* and *CDH10* genes related to ASD disorder on protein structure, function, and stabilization were obtained using *in silico* methods. *CDH8*; rs145143780 (Y572C) and *CDH10*; rs13174039 (V459G), rs147882578 (N485K), rs201423740 (Y306C), rs201956238 (F317L), rs373340564 (R128C) were identified as high-risk SNPs via bioinformatics analysis tools as shown in the workflow in Figure 1 (Table 4). During the creation of three-dimensional models by the Project HOPE, analysis is also performed in terms of hydrophobicity, charge, and size differences. It was also evaluated how the SNPs detected in both genes affect the structure or function of the protein in this respect Table 3. Changes that may occur in protein stabilization can cause proteins to deteriorate or misfold [39]. Due to the decrease in stabilization because of the amino acid change that occurs, folds with low success rates or an increase in unfolded protein sections can be seen [40]. In our study, it was determined that it decreased protein stabilization in all variants that were predicted to have a possible harmful effect. Although it has not yet been defined in autism, which is a ND, current studies show that multiple genes, epigenetic effects, or gene-gene interactions pose a risk [41,42]. Therefore, the gene-gene interactions were determined via GeneMANIA. For example, it was determined that physical and genetic interaction, co-expression, co-localization, estimated interaction, shared protein domains and pathways in *CDH8* and *CDH10* genes (Figure 2) [43].

Table 4. HOPE tool 3-D modelling results of *CDH8* and *CDH10* genes

Gene Name	SNP ID	MODELING	EXPLANATION
CDH8	**rs145143780 (Y572C)**		As a result of the rs145143780 polymorphism, the amino acid tyrosine is converted to cysteine at position 572.
	rs13174039 (V459G)		As a result of the rs13174039 polymorphism, the amino acid valine is converted to glycine at position 459.
	rs147882578 (N485K)		As a result of the rs147882578 polymorphism, the amino acid Asparagine is converted to Lysine at position 485.
CDH10	**rs201423740 (Y306C)**		As a result of the rs201423740 polymorphism, the amino acid Tyrosine is converted to Cysteine at position 306.
	rs201956238 (F317L)		As a result of the rs201956238 polymorphism, the amino acid phenylalanine is converted to leucine at position 317.
	rs373340564 (R128C)		As a result of the rs373340564 polymorphism, the amino acid Arginine is converted to Cysteine at position 128.

In a study conducted on the *CDH8* gene, they reported that *CDH8* showed a predisposition to autism and learning disabilities as a result of rare familial 16q21 microdeletions and expression analysis [6]. In an ASD study in which genetic analysis was performed, including more than 10,000 people of European origin, it was determined that *CDH10* and *CDH9* genes showed a significant relationship in the pathogenesis of ASD [44]. To understand the role of classical Type II cadherins (CDH8 and CDH11*)* in the etiology of ASD, their expression patterns were analyzed during mouse brain development and in autism-specific human tissue (induced pluripotent stem cell (iPSC)). The results show that both cadherins may have a critical role in ASD [45].

5. Conclusions

As a result of this study, high-risk missense SNPs, which are predicted to have potentially harmful effects, were detected in each of the online analysis tools used. In addition to making more cost-effective, target-oriented studies in a shorter time with *in-silico* methods, it also makes a great contribution to providing preliminary information for experimental studies that are planned by making pre-laboratory preliminary studies. In particular, it is predicted that it will be useful in SNP research and genotyping studies on the *CDH8* and *CDH10* genes.

References

[1] Mostafavi M, Gaitanis J. Autism spectrum disorder and medical cannabis: review and clinical experience. Semin Pediatr Neurol. 2020; 35, 100833.
[2] Ozdemir, O. The green tea polyphenol EGCG modulates NGF, BDNF, and CAMKII-α pathways to alleviate neurological damage in autism-induced rats. Acta Pol. Pharm. Drug Res. 2021; 77, 889-895.
[3] Hirota T, King BH. Autism spectrum disorder: A review. Jama. 2023; 329(2), 157-168.
[4] Persico AM, Napolioni V. Autism genetics. Behav. Brain Res. 2013; 251, 95–112.
[5] Myers SM, Challman TD, Bernier R, Bourgeron T, Chung WK, Constantino JN, Eichler EE, Jacquemont S, Miller DT, Mitchell KJ. Insufficient evidence for "autism-specific" genes. The American Journal of Human Genetics. 2020; 106(5), 587–595.
[6] Pagnamenta AT, Khan H, Walker S, Gerrelli D, Wing K, Bonaglia MC, Giorda R, Berney T, Mani E, Molteni M. Rare familial 16q21 microdeletions under a linkage peak implicate cadherin 8 (CDH8) in susceptibility to autism and learning disability. J. Med. Genet. 2011; 48(1), 48–54.
[7] Lin YC, Frei JA, Kilander MBC, Shen W, Blatt GJ. A subset of autism-associated genes regulate the structural stability of neurons. Front. Cell. Neurosci. 2016; 10, 263.
[8] Friedman LG, Riemslagh FW, Sullivan JM, Mesias R, Williams FM, Huntley GW, Benson DL. Cadherin-8 expression, synaptic localization, and molecular control of neuronal form in prefrontal corticostriatal circuits. J. Comp. Neurol. 2015; 523(1), 75–92.
[9] Takeichi M. The cadherin superfamily in neuronal connections and interactions. Nat. Rev. Neurosci. 2007; 8(1), 11-20.
[10] Maître JL, Heisenberg CP. Three functions of cadherins in cell adhesion. Curr. Biol. 2013; 23(14), R626–R633.
[11] Redies C, Hertel N, Hübner CA. Cadherins and neuropsychiatric disorders. Brain Res. 2012; 1470, 130-144.
[12] Ray M, Mishra J, Priyadarshini A, Sahoo S. In silico identification of potential drug target and analysis of effective single nucleotide polymorphisms for autism spectrum disorder. Gene Reports. 2019; 16, 100420.
[13] Avsar O. Investigation of Putative Functional SNPs of Human HAT1 Protein: A Comprehensive "in silico" Study. Cytol. Genet. 2022; 56(1), 98–107.
[14] Kucukkal TG, Petukh M, Li L, Alexov E. Structural and physico-chemical effects of disease and non-disease nsSNPs on proteins. Curr. Opin. Struct. Biol. 2015; 32, 18–24.
[15] Owji H, Eslami M, Nezafat N, Ghasemi Y. In silico elucidation of deleterious non-synonymous SNPs in SHANK3, the autism spectrum disorder gene. J. Mol. Neurosci. 2020; 70, 1649–1667.
[16] Tekcan A. In silico analysis of FMR1 gene missense SNPs. Cell Biochem. Biophys. 2016; 74, 109–127.
[17] Bekisz S, Baudin L, Buntinx F, Noël A, Geris L. In vitro, in vivo, and in silico models of lymphangiogenesis in solid malignancies. Cancers. 2022; 14(6), 1525.
[18] Hoda A, Lika M, Kolaneci V. Identification of deleterious nsSNPs in human HGF gene: in silico approach. J. Biomol. Struct. Dyn. 2023; 41(21), 11889–11903.
[19] Yusuf M. Insights into the in-silico research: current scenario, advantages, limits, and future perspectives. Life in Silico. 2023; 1(1), 13–25.
[20] Arpi MNT, Simpson TI. SFARI genes and where to find them; modelling Autism Spectrum Disorder specific gene expression dysregulation with RNA-seq data. Sci. Rep. 2022; 12(1), 10158.
[21] Li M, He M, Xu F, Guan Y, Tian J, Wan Z, Zhou H, Gao M, Chong T. Abnormal expression and the significant prognostic value of aquaporins in clear cell renal cell carcinoma. PloS One. 2022; 17(3), e0264553.
[22] Montojo J, Zuberi K, Rodriguez H, Kazi F, Wright G, Donaldson SL, Morris Q, Bader GD. GeneMANIA Cytoscape plugin: fast gene function predictions on the desktop. Bioinformatics. 2010; 26(22), 2927–2928.
[23] Ng PC, Henikoff S. SIFT: Predicting amino acid changes that affect protein function. Nucleic Acids Res. 2003; 31(13), 3812–3814.

[24] Adzhubei I, Jordan DM, Sunyaev SR. Predicting functional effect of human missense mutations using PolyPhen-2. Curr. Protoc. Hum. Genet. 2013; 76(1), 7-20.
[25] Pshennikova VG, Barashkov NA, Romanov GP, Teryutin FM, Solov'ev AV, Gotovtsev NN, Nikanorova A A, Nakhodkin SS, Sazonov NN, Morozov IV. Comparison of predictive in silico tools on missense variants in GJB2, GJB6, and GJB3 genes associated with autosomal recessive deafness 1A (DFNB1A). The Scientific World Journal. 2019; 2019.
[26] Choi Y, Chan AP. PROVEAN web server: a tool to predict the functional effect of amino acid substitutions and indels. Bioinformatics. 2015; 31(16), 2745–2747.
[27] Sandell L, Sharp NP. Fitness effects of mutations: An assessment of PROVEAN predictions using mutation accumulation data. Genome Biol. Evol. 2022; 14(1), evac004.
[28] Pawlina-Tyszko K, Semik-Gurgul E, Gurgul A, Oczkowicz M, Szmatoła T, Bugno-Poniewierska M. Application of the targeted sequencing approach reveals the single nucleotide polymorphism (SNP) repertoire in microRNA genes in the pig genome. Sci. Rep. 2021; 11(1), 1–12.
[29] Schwarz DF, Hädicke O, Erdmann J, Ziegler A, Bayer D, Möller S. SNPtoGO: characterizing SNPs by enriched GO terms. Bioinformatics. 2008; 24(1), 146–148.
[30] Capriotti E, Altman RB, Bromberg Y. Collective judgment predicts disease-associated single nucleotide variants. BMC Genomics. 2013;14(3), 1–9.
[31] Petrosino M, Novak, L, Pasquo A, Chiaraluce R, Turina P, Capriotti E, Consalvi V. Analysis and interpretation of the impact of missense variants in cancer. Int. J. Mol. Sci. 2021; 22(11), 5416.
[32] AbdulAzeez S, Borgio JF. In-silico computing of the most deleterious nsSNPs in HBA1 gene. PloS One. 2016; 11(1), e0147702.
[33] Munshani S, Ibrahim, EY, Domenicano I, Ehrlich BE. The impact of mutations in wolframin on psychiatric disorders. Front. Pediatr. 2021; 9, 718132.
[34] Desai M, Chauhan JB. Predicting the functional and structural consequences of nsSNPs in human methionine synthase gene using computational tools. Systems Biology in Reproductive Medicine. 2019; 65(4), 288–300.
[35] Lim SW, Tan KJ, Azuraidi OM, Sathiya M, Lim EC, Lai KS, Yap WS, Afizan NARNM. Functional and structural analysis of non-synonymous single nucleotide polymorphisms (nsSNPs) in the MYB oncoproteins associated with human cancer. Sci. Rep. 2021; 11(1), 1–14.
[36] Tanwar H, Kumar DT, Doss C, Zayed H. Bioinformatics classification of mutations in patients with Mucopolysaccharidosis IIIA. Metab. Brain Dis. 2019; 34(6), 1577–1594.
[37] Sadakierska-Chudy A, Szymanowski P, Lebioda A, Płoski R. Identification and In Silico Characterization of a Novel COLGALT2 Gene Variant in a Child with Mucosal Rectal Prolapse. Int. J. Mol. Sci. 2022; 23(7), 3670.
[38] Venkata Subbiah H, Ramesh Babu P, Subbiah U. In silico analysis of non-synonymous single nucleotide polymorphisms of human DEFB1 gene. Egypt. J. Med. Hum. Genet. 2020; 21(1), 1–9.
[39] Matthews BW, Nicholson H, Becktel WJ. Enhanced protein thermostability from site-directed mutations that decrease the entropy of unfolding. Proc. Natl. Acad. Sci. U.S.A. 1987; 84(19), 6663-6667.
[40] Ragoonanan V, Aksan A. Protein stabilization. Transfus Med Hemother. 2007; 34(4), 246-252.
[41] Ma DQ, Whitehead PL, Menold MM, Martin ER, Ashley-Koch AE, Mei H, Ritchie MD, Delong GR, Abramson RK, Wright HH. Identification of significant association and gene-gene interaction of GABA receptor subunit genes in autism. The American Journal of Human Genetics. 2005; 77(3), 377–388.
[42] Moore JH. The ubiquitous nature of epistasis in determining susceptibility to common human diseases. Hum Hered. 2003; 56(1–3), 73–82.
[43] Warde-Farley D, Donaldson SL, Comes O, Zuberi K, Badrawi R, Chao P, Franz M, Grouios C, Kazi F, Lopes CT. The GeneMANIA prediction server: biological network integration for gene prioritization and predicting gene function. Nucleic Acids Res. 2010; 38(suppl_2), W214–W220.
[44] Wang K, Zhang H, Ma D, Bucan M, Glessner JT, Abrahams BS, Salyakina D, Imielinski M, Bradfield JP, Sleiman PMA. Common genetic variants on 5p14. 1 associate with autism spectrum disorders. Nature. 2009; 459(7246), 528–533.
[45] Frei JA, Niescier RF, Bridi MS, Durens M, Nestor JE, Kilander MBC, Yuan X, Dykxhoorn DM, Nestor M W, Huang S. Regulation of neural circuit development by cadherin-11 provides implications for autism. Eneuro. 2021; 8(4).