In Silico Identification of Possible Mutation-Prone Regions of the GRIA3 Protein

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1. Introduction

Schizophrenia is characterized by positive psychotic symptoms, including hallucinations and delusions, and negative symptoms including emotional limitation and social impairment (Smeland., 2020). There are three symptom groups in schizophrenia; positive, negative and cognitive (Stepnicki P., et al. 2018). It is a chronic disease triggered by the interaction of genetic, epigenetic, developmental and environmental factors that interfere with normal brain development and maturation (Millan M. et al., 2016). Although genetic linkage and association studies have targeted a large number of candidate loci and genes, no specific gene variant or gene combination is sufficient to be shown to cause schizophrenia (Jablensky A., 2022). Currently, schizophrenia cannot be diagnosed with genetic risk factors, but further genetic studies will contribute to the diagnosis of the disease (Smeland., 2020).

1.1.Schizophrenia and GRIA3 Protein

Abnormalities in the expression of iontropic glutamate receptor subunits in the temporal lobe of the brain have been detected in schizophrenia and mood disorders (Beneyto M. et al. 2007). In studies conducted with the brains of patients who died of schizophrenia, it has been reported that the expression of the AMPA receptor is abnormal, but inconsistencies exist as in other theories to schizophrenia (Yonezawa K., et al., 2022). In a comprehensive study investigating rare encoded variants in genes that carry a significant risk for schizophrenia, it was stated that NMDA receptor subunit GRIN2A and AMPA receptor GRIA3 play a role in the pathogenesis of schizophrenia together with the disorder of the glutamatergic system (Singh T., et al, 2022).

1.2. Glutamate Receptors

One of the primary excitatory neurotransmitters and the most prevalent neurotransmitter in mammalian brains is glutamate (Moghaddam B., et al, 2012). Most glutamate receptors are ionotropic receptors with ligand-gated ion channels, but a few metabotropic glutamate receptors are also found. Ionotropic glutamate receptors are divided into three subtypes; NMDA (N-methyl-D-aspartate), AMPA (α-amino-3-hydroxy-5-methylisoxaole-4-propionate), and Kainate receptors (Uno

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Y., et al., 2019 , García-Gaytán AC., et al., 2022). The ionotropic glutamate receptors in the brain play a major role in mediating fast excitatory neurotransmission (Beneyto M. et al.,2007).

1.2.1 NMDA receptors.

NMDA receptors are ligand-gated ion channels that conducts excitatory transmission through a Ca2+ permeable component in the central nervous system. It has important physiological roles such as learning, memory and synaptic flexibility (Hansen KB., et al., 2018). NMDA receptors have 7 subunits; GRIN1, GRIN2 A-D and GRIN3 A-B (García-Gaytán AC., et al., 2022).

1.2.2 AMPA receptors.

Together with the NMDA and Kainat receptors, AMPA receptors are tetrameric ion channels that mediate most of the excitatory transmission in the central nervous system. They are the main fast transmission elements in synapses (Greger IH., et al., 2017). The activity of AMPA receptors is critical not only for neuronal development and synaptic plasticity in physiological conditions, but also because it can induce neuronal death in neuropathological conditions (Guo C., et al., 2021). They are transmembrane proteins that combine as a tetrameric complex consisting of 4 subunits, GRIA 1-4 (Kamalova A., et al., 2021). Disorders of the GRIA3 protein have been associated with disorders such as intellectual developmental disorder, bipolar disorder, schizophrenia, epilepsy, autism, and spasticity (MalaCards).

1.3. Detection of Mutations

Single nucleotide polymorphisms, SNPs, are the most common genetic mutation (Ou L., et al., 2017). SNPs that change the amino acid sequence are called nonsynonymous single nucleotide polymorphisms (nsSNPs). The translation product is affected by the change in the amino acid sequence (Soremekun OS, et al., 2021). Some SNPs can cause disease, predispose a person to disease, or impact how well they respond to medicine, while others are phenotypically neutral. (Ou L., et al., 2017). Single amino acid changes are the most common cause of many diseases. Examining single point mutations of a protein is of great importance for understanding disease mechanisms (Ng PC., et al., 2006). The development of databases and web-based algorithms that calculate the probability of mutations being harmful to see the effect of these mutations on proteins has facilitated and expanded research in this area (Choudjury A., et al., 2022). In silico studies are an important method used in drug discovery and development. Such studies provide advantages in terms of cost as they replace human and animal experiments (Saldanha L., et al., 2023).

In this study, DisEMBL, I-Mutant 2.0 and PhD-SNP bioinformatics tools will be used to detect various mutation-prone regions of the AMPA receptor subunit GRIA3 protein, a protein identified in the literature that may be a possible cause of schizophrenia, and to examine the pathogenic effects of these mutations.

2.Methods & Results

The NCBI-National Center for Biotechnology, the National Center for Biotechnology Information, is a platform established to provide access to biomedical and genomic information

(NCBI-National Center for Biotechnology). In order to reach the GRIA3 protein via NCBI, "Glutamate Receptor 3" was searched in "Homo sapiens" was selected from the species filter for sequence information in humans.

The code representing the protein is P42263.2 (NCBI, UniProtKB). The FASTA format is a text format representing nucleotide or peptide sequences with the code in which base pairs or amino acids are represented by single letters (NCBI-National Center for Biotechnology). The FASTA sequence of the protein at the amino acid level obtained is as follows.

>sp|P42263.2|GRIA3_HUMAN RecName: Full=Glutamate receptor 3; Short=GluR-3; AltName: Full=AMPA-selective glutamate receptor 3; AltName: Full=GluR-C; AltName: Full=GluR-K3; AltName: Full=Glutamate receptor ionotropic, AMPA 3; Short=GluA3; Flags: Precursor

MARQKKMGQSVLRAVFFLVLGLLGHSHGGFPNTISIGGLFMRNTVQEHSAFRFAVQLYNTNQNTTEKPFHLNYHVDHL
DSSNSFSVTNAFCSQFSRGVYAIFGFYDQMSMNTLTSFCGALHTSFVTPSFPTDADVQFVIQMRPALKGAILSLLGHYKWE
KFVYLYDTERGFSILQAIMEAAVQNNWQVTARSVGNIKDVQEFRRIIEEMDRRQEKRYLIDCEVERINTILEQVVILGKH
SRGYHYMLANLGFTDILLERVMHGGANITGFQIVNNENPMVQQFIQRWVRLDEREFPEAKNAPLKYTSALTHDAILVIA
EAFRYLRRQRVDVSRRGSAGDCLANPAVPWSQGIDIERALKMVQVQGMTGNIQFDTYGRRTNYTIDVYEMKVSGSRKA
GYWNEYERFVPFSDQQISNDSASSENRTIVVTTILESPYVMYKKNHEQLEGNERYEGYCVDLAYEIAKHVRIKYKLSIVG
DGKYGARDPETKIWNGMVGELVYGRADIAVAPLTITLVREEVIDFSKPFMSLGISIMIKKPQKSKPGVFSFLDPLAYEIWM
CIVFAYIGVSVVLFLVSRFSPYEWHLEDNNEEPRDPQSPPDPPNEFGIFNSLWFSLGAFMQQGCDISPRSLSGRIVGGVWWF
FTLIIISSYTANLAAFLTVERMVSPIESAEDLAKQTEIAYGTLDSGSTKEFFRRSKIAVYEKMWSYMKSAEPSVFTKTTADGV
ARVRKSKGKFAFLLESTMNEYIEQRKPCDTMKVGGNLDSKGYGVATPKGSALRNAVNLAVLKLNEQGLLDKLKNKW
WYDKGECGSGGGDSKDKTSALSLSNVAGVFYILVGGLGLAMMVALIEFCYKSRAESKRMKLTKNTQNFKPAPATNTQN
YATYREGYNVYGTESVKI

2.1.Identification of Mutation-Prone Regions of the GRIA3 Protein

Using the amino acid sequence of GRIA3, mutation-prone regions in the GRIA3 protein were investigated by estimating the irregular/unstructured regions, then stability changes in these regions, evolutionary analysis, and SNP estimation.

2.2.Prediction of disordered/unstructured regions of the GRIA3 protein

The tool DisEMBL, was used to predict the disordered/unstructured regions in the sequence of the GRIA3 protein. With the DisEMBL tool, irregular regions can be examined for Loop and Coil, Hot-loops and Remark-465 (Linding R., et al., 2003).

Estimations were obtained by loading the amino acid sequence of the GRIA3 protein into the DisEMBL tool and converted into a table as in Table 1. In terms of loops/coils, the irregular/unstructured regions were estimated as 1-8, 390-422, 793-817. Irregular/unstructured regions in terms of hot cycles were estimated as 1-10, 409-424, 805-813. In terms of Remark-465, irregular/unstructured regions were estimated as 1-8, 412-421, 806-815. When the regions common in all results were combined, MARQKKMG between regions 1 and 8, QISNDSASSE between regions 412 and 421, and GSGGGDSK between regions 806 and 813 were determined as common. These detected regions will be used in the next stages of the study. Regions and amino acids determined to be common in the results obtained in Table 1 are highlighted in bold.

Table 1. Results of disordered/unstructured regions of the GRIA3 protein predicted by the DisEMBL tool.

Estimation tool DisEMBL	**Estimated Locations**	**Common Predicted Results**
Disordered by Loops/coils definition	**1-8**, 24-44, 59-86, 119-134, 262-278, 290-308, 329-352, 366-381, **390-422**, 442-453, 472-491, 500-507, 534-549, 575-606, 617-634, 661-668, 678-691, 707-718, 744-773, **793-817**, 852-894	**MARQKKMG: 1-8** **QISNDSASSE: 412-421**
Disordered by Hot-loops definition	**1-10**, 190-200, 270-278, 298-310, 330-349, 367-378, **409-424**, 429-437, 470-489, 534-542, 678-691, 705-726, 791-800**, 805-813**, 859-882	**GSGGGDSK: 806-813**
Disordered by Remark-465 definition	**1-8, 412-421**, 585-597, **806-815**	

2.3.Estimation of stability changes of the GRIA3 protein

The I-Mutant 2.0 tool was used to examine the effect of single point mutations on protein stability in the construct and sequence. After loading the amino acid sequence of the protein to the site, the position of the single point mutations was written and the new residue part was left blank, and the stability change of all 19 amino acid changes was obtained. The temperature in degrees Celsius was adjusted to 37 and the pH (-log[H+]) to 7.4 (Capriotti E., et al., 2005). Protein stability, which is changed by a single point mutation, is given by the score obtained from the protein's structure information and sequence information. The RI value is the Reliability Index.

Of the 475 results obtained by performing 19 single point mutations of 25 amino acids, only those with a confidence index above 8 are selected and shown in Table 2. The RI value of 35 results is 8. Of the 32 stability change analyzes with an RI value of 8 and above, 23 results showed a decrease in stability, while 9 results showed an increase in stability.

Table 2. Effect of GRIA3 mutations predicted by the I-Mutant 2.0 tool on protein stability

Estimation tool	**Variant**	**Results**	**RI**	**Variant**	**Results**	**RI**	**Variant**	**Results**	**RI**
I-Mutant	R3G	Decrease	8	I413C	Decrease	8	G808R	Decrease	8
	K5P	Increase	8	N415G	Decrease	8	G808K	Decrease	8
	K5L	Increase	8	N415A	Decrease	8	G809K	Decrease	8
	K6L	Increase	8	A418Y	Decrease	8	G809Q	Decrease	8
	K6V	Increase	8	A418K	Decrease	8	G809D	Decrease	8
	K6P	Increase	8	A418T	Decrease	8	G809R	Decrease	8
	K6F	Increase	8	A418Y	Decrease	8	G810Q	Decrease	8
	M7H	Decrease	8	E421A	Decrease	8	S812A	Decrease	8
	G8K	Decrease	8	E421K	Decrease	8	K813A	Decrease	8
	I413E	Decrease	8	E421Q	Decrease	8			

pH.:7.4, Temperature:37

2.4.Prediction of SNPs of GRIA3 protein

PhD-SNP is an optimized tool to predict whether a given single point protein mutation can be classified as a disease-associated or neutral polymorphism (Capriotti E., et al., 2006). The analysis was performed by entering the protein sequence, the location of the mutation, and the symbol of the new amino acid in the tool, replacing all 19 amino acids for 25 positions. As a result of the analysis, the disease effect of the mutation and the Relativity Index(RI) value was obtained. Disease results among the results obtained are given in Table 3.

Table 3. Disease effect of GRIA3 mutations estimated by the PhD-SNP tool

Estimation Tool	**Variant**	**Results**	**RI**	**Variant**	**Results**	**RI**	**Variant**	**Results**	**RI**
PhD -SNP	M7P	Disease	1	D416C	Disease	3	G808C	Disease	3
	M7K	Disease	1	D416H	Disease	2	G808P	Disease	1
	Q412P	Disease	4	D416K	Disease	1	G809C	Disease	2
	I413P	Disease	2	S417I	Disease	3	G810C	Disease	1
	S414P	Disease	2	S417F	Disease	0	D811V	Disease	1
	N415V	Disease	1	S417W	Disease	2	D811F	Disease	2
	N415L	Disease	2	S417K	Disease	2	D811W	Disease	1
	N415F	Disease	3	S417E	Disease	0	D811Y	Disease	5
	N415W	Disease	3	S419P	Disease	1	D811A	Disease	3
	N415T	Disease	2	G806Y	Disease	0	D811C	Disease	3
	N415Q	Disease	1	G806C	Disease	2	D811H	Disease	1
	N415R	Disease	3	S807W	Disease	0	D811R	Disease	2
	N415K	Disease	2	S807Y	Disease	2	D811Q	Disease	1
	D416Y	Disease	2	S807R	Disease	2	S812W	Disease	0
	D416P	Disease	4						

3.Discussion

The disease effect and stability changes of possible single amino acid changes of the GRIA3 protein associated with schizophrenia were investigated using bioinformatics tools such as DisEMBL, I-Mutant 2.0, and PhD-SNP. Many disease results were estimated in N415, D416, S417 and D811 reigons, highlighting these region's importance. The results obtained enabled the identification of potential variants that may cause schizophrenia. The results of this study can be supported by different in silico studies to be done later.

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