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IN – SILICO ANALYSIS OF SNPS AND 3'UTR ASSOCIATED MIRNAS IN GENES FOR STUDYING THE PATHOGENESIS OF EPILEPSY

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Abstract— Epilepsy is a neurological disorder. It is not a communicable disease . In this disease the nerve cell activity get disturbed in the brain. It may be caused due due to the genetic disorder or any kind of brain injury.
Different bioinformatics tools (online servers) were used to foretell the effect of the SNP on structure and functioning of the proteins used here. miRNA founded in the UTR regions of the proteins (GABARAP ZDHHC3 NLGN2) were also defined .
The current study illustrates that rs2134510, rs367707073, and rs372747001 for GABARAP protein, rs150545990, rs201481724, rs201972977, and rs376668595 for NLGN2 gene and rs140002813, rs143591132 and rs376006143 for ZDHHC3 may have role in the disease. Thus these mutations might be responsible for the pathophysiology of the disease.

Index Terms- Epilepsy , GABARAP gene , NLGN2 gene , ZDHHC3 gene , SNPs

Introduction

Epilepsy is (non communicable & chronic) neurological disorder of brain malfunction, characterized by recurrent seizures [1]. The disorder is most common among neurological disorder affect 50 million people globally [20]. The exact reason behind the disorder remains unclear but some of the causes include lack of oxygen during birth, brain stroke, brain malformation, brain tumor and excess use of alcohol [2]. Besides these factors, genetic factors also played a vital role in pathophysiology of the disorder. Twin studies indicated that twins monozygotic in nature have four times more risk of causing epilepsy than of dizygotic twins [3]. The symptoms of epilepsy include shivering, headache, anxiety, jerking movements of legs and arms (uncontollabe), temporary confusion , fear [20]. Patients with epilepsy often displays the pathological changes in the brain's activity between the seizure events which is called as Interictal Epileptiform Discharges (IEDs). Biological rhythms like menstrual cycle , lunar phase , multidien rhythms , circadian rhythms influence the seizure associated death and the timing of the seizure [21]. It is been estimated that most of the epilepsies have a genetic (base) basis [5]. Genes like potassium voltage-gated channel subfamily Q, sodium voltage-gated channel alpha subunit 1, and cholinergic receptor nicotinic alpha 4 subunit have been known to contribute to the disease [4]. Epilepsies having abnormalities (genetic) exhibit heterogeneity in large amount. In some genes mutations may be associated with the pathophysiology of epilepsy (e.g., mutations in genes SCN1A, TSC1 and TSC2) [6]. There are many genes known, mutations in those genes are responsible for the occurrence of disease. Previous studies have provided evidence of (γ-aminobutyric acid) GABA in epilepsy. GABA is one of the crucial inhibiting neurotransmitter of the mammalian CNS (central nervous system). There are two classes of GABA receptors, ligand operated ion channels $GABA_A$ receptors and $GABA_B$ receptor which is G- protein coupled metabotropic receptors [9]. GABAergic neurons are distributed all over the brain and has a essential role in processing and integration of neuronal functions.

GABARAP (GABA associated protein) is mainly located in Golgi apparatus, showing its major role in intracellular trafficking of GABA [10]. It binds with intracellular domain of γ-2 subunit of GABA in vitro and in vivo [11, 12], because of mutation in C-terminal (G116A), cleavage of C terminal of GABARAP can be blocked, which could disfigure the phospholipids addition to GABARAP which is very important in controlling the trafficking of GABA [13].

NLGN2 stands for NEUROLIGIN – 2 which is a protein in humans. It has a number of molecular functions like signalling

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receptor activity , cell adhension molecule binding , identical protein binding etc. involvement of the members of this family is also found in the remodeling and formation of synapses of (central nervous system) CNS.

ZDHHC3 – (PALMITOYLTRANSFERASE) does a number of molecular functions such as transferase activity , protein – cysteine S – palmitoyl transferase activity etc. . ZDHHC3 is responsible for number of biological processes like localization within membrane, protein palmitoylation, protein targeting , protein localization to photoreceptor outer segment , protein targeting to membrane.

SNPs are genetic variation of most common type among human beings. It stands for SINGLE NUCLEOTIDE POLYMORPHISM also pronounced as snips. Difference in the single nucleotide (DNA building blocks) is represented by each SNP . they can be found in coding region but mostly found in the non-coding regions . there are two types of SNPs i.e. non- synonymous and synonymous ,fund in the coding region . Non – synonymous is further divided into two types i.e. missense and nonsense .SNPs can act like a biological markers which help to locate genes associated with disease . they are useful in the study of drug resistance in epilepsy .it can be utilized in identifying disorder causing genes in human beings. We can also recognize molecular mechanisms of sequence evolution .

The aim of this analysis is to provide an intuition into those genes that should be included in (genetic) testing of the patients having distinct phenotype. The relation between epilepsy and genes will further inhance our knowledge on the role playacted by specific mutations in epilepsy and mechanisms fundamental to epileptogenesis [8].

Material and methods :

1.1 Retreiving nsSNPs

Non-synonymous SNPs (nsSNPS) were fetched from database dbSNP. It is a large database of nucleotide sequence variations in genetic material. NCBI (National Centre for Biotechnology Information) maintains the dbsnp. It is a free public archive containing genetic information of various species. It was created in September 1998 to supplement GenBank. It is a collection of databases including: each individual species and a range of molecular variations like, microsatellite markers or short tandem repeats (STRs) , SNPs , short deletion and insertion polymorphisms (indels / DIPs) , multimucleotide polymorphism 9 MNPs) , named varients , heterozygous sequences. The natural variants and sequence of ZDHHC3, NLGN2 and GABARAP proteins were drawn from UniProt database (online tool) as it is considered most unambiguous and reliable database for protein sequences [14] (https://www.uniprot.org/).

Total(number) of 1630, 4791and 14462 nsSNPs were found from NCBI database for GABARAP, NLGN2 and ZDHHC3, respectively all of them were then subjected to the in silico analysis using 10 different (servers)softwares and algorithms; namely SIFT, PROVEAN, PolyPhen-2, SNPs & GO, I-mutant, ,GeneMANIA and Chimera.

1.2 Identifying the most damaging nssnpa and disease related mutations

1.2.1 Sift (Sorting Intolerant From Tolerant)

Sorting Intolerant From Tolerant (SIFT) server was used for detecting phenotypic effects of the amino acid substitution on the protein function, this server is powerful (strong)tool used to accomplish this purpose (http://sift.bii.a-star.edu.sg/). A list of (rsIDs) nsSNPs from the NCBI's dbSNP database were submitted as (original) query sequences to the SIFT for predicting deleterious and tolerated substitutions for every position of protein sequence. The server provides the results in two i.e."Deleterious" &"Tolerated", nsSNPs with SIFT score ≤ 0.05 were classified as deleterious and are then further analysis is done for identifying the damaging ones, and those which were > 0.05 were classified as tolerated and they are then not further analyzed[15] (Sim et al., 2012).

1.*2.2 Provean*

(Protein Variation Effect Analyzer) PROVEAN is the second (server) software tool used (http://provean.jcvi.org/index.php). It also depictsts effect of an amino acid substitution on biological functioning of the protein. It is used to predict damaging effects of any type of protein sequence variations to not only single amino acid substitutions, but also in-frame insertions, deletions, multiple amino acid substitutions. The results are obtained as either "Deleterious" if prediction score was <-2.5, while score > −2.5 indicates that variant is predicted to have a "Neutral" effect (Kumar et al., 2018).

1.2.3 Snp & go

It is an online tool (web server) software which is used for ensuring the disorder relation with the studied (Single Nucleotide Polymorphisms) SNPs (http://snps-and-go.biocomp.unibo.it/snps-and-go/). It provides 3 different results depending on 3 different analytical algorithms; Panther results, PHD-SNP results, and SNPs & GO result. Each of these obtained results containing of 3 parts, having prediction deciding whether the mutation is neutral or related to disorder , reliability index (RI), and disorder probability (if disorder probability is >0.5 then mutation is regarded as a disorder causing nsSNP) [17].

1.3 Protein structural analysis :

I Mutant

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An online webserver which is used for predicting the protein stability change upon single point mutation, used to determine protein stability whether - mutation increase or decrease protein's stability (http://gpcr.biocomp.unibo.it/cgi/predictors/I-Mutant2.0/I-Mutant2.0.cgi) [18].

1.4 Genemania:

A web server- tool used to know the protein functions, analyze submitted gene list and then prioritize genes for the functional assays. It provides lists of the genes which are functionally similar. It makes identifications and recognitions using available proteomics , genomics data. (http://www.genemania.org/)[19].

1.5 3' UTR analysis :

1.5.1 Ensemble 95

RNA sequences were recovered from Ensemble, it is a webserver that provides key genomic data sets) to the scientific community without any kind of restrictions.

1.5.2 RegRNA2.0

Then , RNA sequences which were isolated using ensemble were then inserted to RegRNA 2.0 (which is an integrated webserver for identifying the functional RNA sites and the motifs).

1.5.3 miRmap22.1

miRNA sequences were then further loaded into miRmap website for predicting the miRNA targets and for studying the repression strength using the thermodynamic, evolutionary, probabilistic, and sequence based approaches. There were no targets found for any of the gene used.

1.5.4 mirbase

After mirmap we used mirbase which is used to identify the nature if the strand (forward or backward) and the assersion number using the mir numbers of the genes used .

Result and discussion

1630, 4791, 14462(SNPs) were retrieved using National Centre for Biotechnology Information (NCBI) website against GABARAP, NLGN2, ZDHHC3 respectively (Fig. 1) and were submitted to SIFT (Fig. 2), Provean, etc. After analysis common deleterious mutations were found using three servers. Table 1 shows the SIFT analysis of all the three genes.

Furthermore, the SNPs were also submitted to provean and SNP&GO (Table 2 and 3) for studying the relation between SNPs and disorder. In case of GABARAP three SNPs were detected to be(damaging)deleterious with all the three severs, five and three SNPs were found deleterious in NLGN2 and ZDHHC3 genes respectively. The SNPs which were found common by all the three servers were studied further for the analysis. rs2134510, rs367707073, and rs372747001 for GABARAP protein were predicted to be deleterious and SNPs rs139254633, rs150545990, rs201481724, rs201972977, and rs376668595 for NLGN2 gene were damaging. For ZDHHC3 SNPs rs140002813, rs143591132 and rs376006143 were deleterious and may have associated with the disease. Then I-mutant was used for detecting stability of the protein and result showed that all three deleterious SNPs of GABARAP, one SNP (rs201481724) for NLGN2 and three SNPs for ZDHHC3 lead to instability of the protein followed by unstable amino acid interaction as shown in Table4.

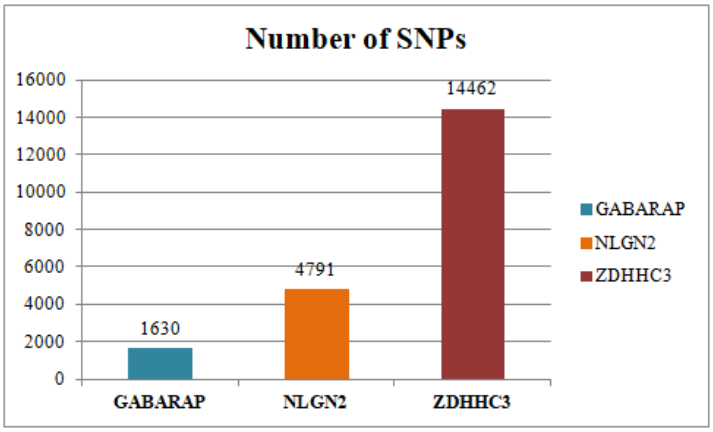

Fig 1 . Graph predicting total number of SNPs

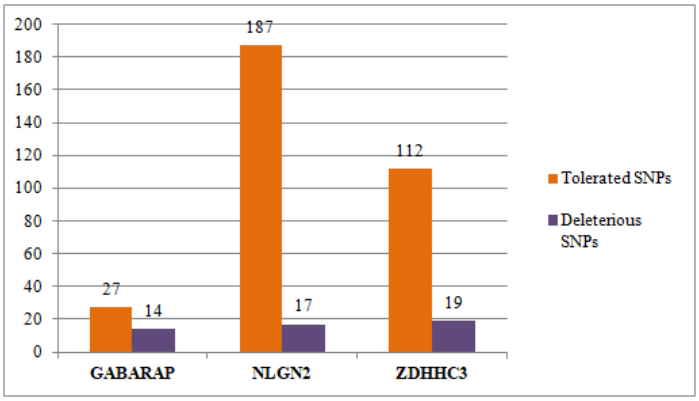

Fig 2 . Graph predicting tolerated and deleterious SNPs .

Table 1: SIFT result of GABARAP, NLGN2 and ZDHHC3 genes

S NO.	SNP	CHR	REFA	ALTAL	AMINO ACID CHANGE	SIFT SCORE	SIFT PREDICTION

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			LLELE	LELE			
GABARAP							
1	rs1802217	17	G	T	R67R	1	TOLERATED
2	**rs2134510**	17	A	G	V33A	0.029	DELETERIOUS
3	rs115012444	17	G	A	S8S	1	TOLERATED
4	rs142077609	17	G	A	S232S	1	TOLERATED
5	rs200698534	17	G	A	A18A	1	TOLERATED
6	rs201373066	17	C	T	R15H	0.58	TOLERATED
7	**rs367707073**	17	T	A	E73V	0.005	DELETERIOUS
9	**rs370491385**	17	C	T	R231H	0.001	DELETERIOUS
10	rs371164291	17	C	T	L234L	1	TOLERATED
11	**rs372747001**	17	G	A	H69Y	0.012	DELETERIOUS
12	rs374949545	17	T	C	Q240Q	0.692	TOLERATED
13	rs376821360	17	G	A	Y61Y	0.626	TOLERATED
14	**rs377543259**	17	A	G	L110P	0.022	DELETERIOUS
NLGN2							
1	rs139407430	17	G	T	L707L	0.365	TOLERATED
2	rs141186472	17	G	A	P630P	1	TOLERATED
3	rs145456351	17	C	T	D527D	1	TOLERATED
4	rs145633225	17	C	T	G444G	0.283	TOLERATED
5	rs150207176	17	G	A	P166P	0.801	TOLERATED
6	rs182557801	17	G	A	T76T	0.592	TOLERATED
7	rs2241233	17	C	T	S176S	0.868	TOLERATED
8	rs12947017	17	C	T	G381G	1	TOLERATED
9	rs61736089	17	G	A	A20A	1	TOLERATED
10	rs62061174	17	C	T	A755V	0.314	TOLERATED
11	rs62061175	17	C	T	A819A	1	TOLERATED
12	rs76733190	17	C	T	P511P	1	TOLERATED
13	rs78081080	17	C	T	P657P	1	TOLERATED
14	rs78355381	17	T	C	S246S	0.647	TOLERATED
15	rs113373567	17	A	G	D358G	0.059	TOLERATED
16	rs114377334	17	G	A	P644P	1	TOLERATED
17	rs116990598	17	C	T	Y376Y	1	TOLERATED
18	rs138117058	17	C	T	R594R	1	TOLERATED
19	rs138187857	17	G	A	P552P	1	TOLERATED
20	rs138726680	17	G	A	G191G	1	TOLERATED
21	**rs139254633**	17	C	T	R331W	0.013	DELETERIOUS
22	rs139552861	17	C	G	A5P	0.276	TOLERATED
23	rs140045012	17	G	A	A453A	1	TOLERATED
24	rs140208582	17	G	A	D440N	0.061	TOLERATED
25	rs140406892	17	A	G	K561R	0.18	TOLERATED
26	rs140489941	17	G	A	A515T	0.2	TOLERATED
27	rs141813037	17	G	T	E174D	0.633	TOLERATED
28	rs142399350	17	G	A	V510M	0.199	TOLERATED
29	rs142497106	17	C	T	A472A	1	TOLERATED
30	rs143644156	17	C	T	P256P	1	TOLERATED
31	rs144019667	17	A	G	N610D	0.119	TOLERATED
32	rs144434883	17	C	T	N412N	1	TOLERATED
34	rs145515125	17	A	G	N592S	0.115	TOLERATED
35	rs145673268	17	C	T	G716G	0.594	TOLERATED
36	rs147358626	17	A	T	P659P	1	TOLERATED
37	rs147515939	17	C	T	Y307Y	1	TOLERATED
38	rs147711069	17	T	C	Q16Q	1	TOLERATED
39	rs147720531	17	T	C	T467T	1	TOLERATED
40	rs149521836	17	C	T	L604L	1	TOLERATED
41	**rs150545990**	17	C	T	R335W	0.005	DELETERIOUS
42	rs199509090	17	T	C	N443N	1	TOLERATED
43	rs199785292	17	C	T	F196F	0.486	TOLERATED
44	rs199983625	17	C	T	D358D	1	TOLERATED
45	**rs200055506**	17	C	T	H612Y	0.018	DELETERIOUS
46	**rs200438064**	17	C	T	R642W	0.015	DELETERIOUS
47	rs200595403	17	G	A	L783L	0.326	TOLERATED
48	**rs201481724**	17	G	C	G186R	0.001	DELETERIOUS
49	rs201493646	17	C	T	R55W	0.105	TOLERATED
50	rs201523501	17	G	A	G754R	0.437	TOLERATED
51	rs201659845	17	G	A	V391V	1	TOLERATED

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							D
52	rs201917326	17	G	A	R26H	0.317	TOLERATED
53	**rs201972977**	17	C	T	T674M	0.009	DELETERIOUS
54	rs367879051	17	G	A	R173Q	0.722	TOLERATED
55	rs367900044	17	G	A	P645P	1	TOLERATED
56	rs368199205	17	C	T	L144L	1	TOLERATED
57	rs368243301	17	C	A	P653T	0.187	TOLERATED
58	rs368300488	17	C	A	P104P	1	TOLERATED
59	rs368402918	17	C	G	P154R	0.354	TOLERATED
60	rs368545471	17	C	T	G3R	0.349	TOLERATED
61	rs368710674	17	C	T	P638L	0.055	TOLERATED
62	rs368724018	17	C	A	L518I	0.133	TOLERATED
63	rs369949476	17	C	T	A489V	0.199	TOLERATED
64	rs370003398	17	G	C	T674T	1	TOLERATED
65	rs370370449	17	G	A	P4L	0.681	TOLERATED
66	rs370535198	17	C	T	A758A	0.822	TOLERATED
67	rs370899257	17	G	A	L602L	0.416	TOLERATED
68	rs370906570	17	G	A	V134M	0.134	TOLERATED
69	rs371020542	17	C	G	D172E	0.951	TOLERATED
70	rs371291629	17	T	C	L70L	1	TOLERATED
71	rs371423406	17	C	T	A162V	0.301	TOLERATED
72	rs371633468	17	G	A	E190E	0.618	TOLERATED
73	rs371672267	17	G	A	R664H	0.124	TOLERATED
74	rs372528711	17	C	T	P653P	1	TOLERATED
75	rs372656072	17	A	G	E577G	0.249	TOLERATED
76	rs373049409	17	G	A	E247K	0.288	TOLERATED
77	rs373212332	17	C	T	D255D	1	TOLERATED
78	rs373215973	17	C	T	S264S	1	TOLERATED
79	rs373371274	17	C	A	T679T	1	TOLERATED
80	rs373919395	17	C	T	R492W	0.16	TOLERATED
81	rs373956106	17	C	T	I349I	1	TOLERATED
82	rs374724532	17	C	A	P361P	1	TOLERATED
83	rs374986007	17	C	A	D668E	1	TOLERATED
84	rs375685202	17	G	A	A489A	0.34	TOLERATED
85	rs375833863	17	G	A	A755A	0.787	TOLERATED
86	rs376519239	17	C	T	A732V	0.188	TOLERATED
87	rs376556572	17	C	T	D778D	1	TOLERATED
88	**rs376668595**	17	A	G	Y418C	0.003	DELETERIOUS
89	rs377106671	17	G	A	E257K	0.647	TOLERATED
90	rs377431497	17	G	A	T620T	1	TOLERATED
91	rs377690737	17	G	T	R55L	0.523	TOLERATED
ZDHHC3							
1	rs367543249	3	C	G	S240T	0.154	TOLERATED
2	rs367543250	3	G	A	I49I	0.689	TOLERATED
3	rs367543251	3	T	C	Y29C	0.177	TOLERATED
4	rs3087823	3	A	C	H220Q	0.338	TOLERATED
5	rs11547235	3	A	G	T5A	0.826	TOLERATED
6	rs35142429	3	G	A	D101D	1	TOLERATED
7	rs62242616	3	G	A	D163D	1	TOLERATED
8	**rs140002813**	3	T	C	M1V	0.046	DELETERIOUS
9	rs140195587	3	C	T	R117H	0.159	TOLERATED
10	rs140839510	3	A	G	E8G	0.073	TOLERATED
11	rs142704157	3	C	T	T100T	1	TOLERATED
12	rs142928618	3	G	A	N79N	1	TOLERATED
13	rs142993665	3	C	T	S230S	1	TOLERATED
14	rs143031574	3	T	C	E17E	1	TOLERATED
15	**rs143591132**	3	G	A	M1I	0	DELETERIOUS
16	rs144475793	3	C	T	A9V	0.063	TOLERATED
17	rs145026580	3	T	C	I27V	0.541	TOLERATED
18	**rs146928995**	3	T	A	I14N	0	DELETERIOUS
19	rs147965604	3	T	C	H220R	0.44	TOLERATED
20	rs148007027	3	C	T	V74M	0.193	TOLERATED
21	rs148855138	3	G	A	P130P	1	TOLERATED
22	rs149405044	3	G	C	L74L	1	TOLERATED
23	rs150873824	3	T	C	P21P	1	TOLERATED
24	rs183712112	3	C	T	V103V	0.95	TOLERATED
25	rs184764141	3	T	G	P180P	0.369	TOLERATED

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26	rs189119439	3	C	T	E187K	0.167	TOLERATED
27	rs191549837	3	G	A	D186D	1	TOLERATED
28	rs199968603	3	C	T	E59E	1	TOLERATED
29	rs200698738	3	G	A	S230L	0.761	TOLERATED
30	**rs200799189**	3	A	C	V168G	0.047	DELETERIOUS
31	rs201609037	3	T	C	M164V	0.312	TOLERATED
32	rs201721944	3	G	A	K11K	1	TOLERATED
33	rs202178574	3	A	G	Y57Y	1	TOLERATED
34	rs367843031	3	A	G	W259R	0.551	TOLERATED
35	**rs368475280**	3	G	A	R148W	0	DELETERIOUS
36	rs368845031	3	G	A	T174T	0.626	TOLERATED
37	rs369925964	3	G	C	P225P	0.493	TOLERATED
38	rs370094075	3	G	A	N11N	0.128	TOLERATED
39	rs370592102	3	T	G	Y57S	0.097	TOLERATED
40	rs371409476	3	T	C	Q285Q	1	TOLERATED
41	rs371820396	3	C	A	V33L	0.763	TOLERATED
42	rs371904572	3	C	T	S170S	0.684	TOLERATED
43	rs373185391	3	G	A	A92A	1	TOLERATED
44	rs373663699	3	C	A	A260S	0.666	TOLERATED
45	rs373752597	3	G	A	N10N	1	TOLERATED
46	rs374546881	3	A	G	D49D	1	TOLERATED
47	**rs374733873**	3	G	A	R14W	0.003	DELETERIOUS
48	rs374804123	3	C	T	A46T	0.221	TOLERATED
49	rs375510763	3	C	T	G123E	0.121	TOLERATED
50	**rs376006143**	3	T	A	I115F	0.033	DELETERIOUS
51	rs376522016	3	C	T	R166Q	0.594	TOLERATED
52	rs376614154	3	C	T	S7S	1	TOLERATED
53	rs377132916	3	G	T	A92D	0.108	TOLERATED
54	rs377350770	3	C	T	P294P	1	TOLERATED
55	**rs377465830**	3	C	G	G44A	0	DELETERIOUS

Table 2: Provean result of GABARAP, NLGN2 and ZDHHC3 genes

S. NO.	POSITION	REF	ALT	SCORE	PREDICTION (CUTOFF=2.5)
			GABARAP		
1	67	R	R	0	Neutral
2	33	V	A	-3.02	Deleterious
3	115	Y	Y	0	Neutral
4	232	S	S	0	Neutral
5	108	A	A	0	Neutral
6	15	R	H	-2.79	Deleterious
7	73	E	V	-6.85	Deleterious
8	231	R	H	-4.81	Deleterious
9	234	L	L	0	Neutral
10	69	H	Y	-3	Deleterious
11	240	Q	Q	0	Neutral
12	61	Y	Y	0	Neutral
13	243	L	P	-1.25	Neutral
			NLGN2		
1	707	L	L	0	Neutral
2	630	P	P	0	Neutral
3	527	D	D	0	Neutral
4	444	G	G	0	Neutral
5	166	P	P	0	Neutral
6	76	T	T	0	Neutral
7	322	S	S	0	Neutral
8	381	G	G	0	Neutral
9	35	A	A	0	Neutral
10	755	A	V	-0.13	Neutral
11	819	A	A	0	Neutral
12	511	P	P	0	Neutral
13	511	P	P	0	Neutral
14	657	P	P	0	Neutral
15	246	S	R	-1.93	Neutral
16	246	S	S	0	Neutral
17	358	D	G	-5.21	Deleterious
18	644	P	P	0	Neutral
19	376	Y	Y	0	Neutral
20	594	R	R	0	Neutral
21	552	P	P	0	Neutral
22	191	G	G	0	Neutral
23	331	R	W	-3.74	Deleterious
24	5	A	P	-1.25	Neutral
25	453	A	A	0	Neutral
26	440	D	N	-3.08	Deleterious
27	561	K	R	-1.28	Neutral
28	515	A	T	-0.48	Neutral
29	320	E	D	-0.9	Neutral
30	510	V	M	-1.63	Neutral
31	472	A	A	0	Neutral
32	256	P	P	0	Neutral
33	610	N	D	-2.1	Neutral
34	412	N	N	0	Neutral
35	716	G	G	0	Neutral
36	659	P	P	0	Neutral
37	307	Y	Y	0	Neutral
38	31	Q	Q	0	Neutral
39	467	T	T	0	Neutral
40	604	L	L	0	Neutral
41	335	R	W	-4.66	Deleterious
42	443	N	N	0	Neutral
43	196	F	F	0	Neutral
44	358	D	D	0	Neutral
45	612	H	Y	-1.83	Neutral
46	642	R	W	-2.44	Neutral
47	783	L	L	0	Neutral
48	186	G	R	-6.53	Deleterious
49	55	R	W	-5.97	Deleterious
50	754	G	R	-0.17	Neutral
51	391	V	V	0	Neutral

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52	172	R	H	-1.5	Neutral
53	674	T	R	-3.4	Deleterious
54	319	R	Q	-0.51	Neutral
55	645	P	P	0	Neutral
56	653	P	T	0.74	Neutral
57	104	P	P	0	Neutral
58	154	P	R	-0.51	Neutral
59	638	P	L	-1.46	Neutral
60	518	L	I	-1.14	Neutral
61	489	A	V	-1.64	Neutral
62	674	T	T	0	Neutral
63	758	A	A	0	Neutral
64	602	L	L	0	Neutral
65	134	V	M	-1.05	Neutral
66	318	D	E	-0.8	Neutral
67	162	A	V	-0.08	Neutral
68	664	R	H	0	Neutral
69	653	P	P	0	Neutral
70	577	E	G	-1.53	Neutral
71	255	D	D	0	Neutral
72	255	D	D	0	Neutral
73	679	T	T	0	Neutral
74	492	R	W	-3.05	Deleterious
75	349	I	I	0	Neutral
76	361	P	P	0	Neutral
77	668	D	E	-0.32	Neutral
78	489	A	A	0	Neutral
79	755	A	A	0	Neutral
80	732	A	V	-0.9	Neutral
81	778	D	D	0	Neutral
82	418	Y	C	-6.53	Deleterious
83	620	T	T	0	Neutral
84	55	R	L	-4.83	Deleterious
ZDHHC3					
1	707	L	L	0	Neutral
2	527	D	D	0	Neutral
3	444	G	G	0	Neutral
4	166	P	P	0	Neutral
5	76	T	T	0	Neutral
6	322	S	S	0	Neutral
7	381	G	G	0	Neutral
8	35	A	A	0	Neutral
9	755	A	V	-0.13	Neutral
10	819	A	A	0	Neutral
11	511	P	P	0	Neutral
12	511	P	P	0	Neutral
13	657	P	P	0	Neutral
14	246	S	R	-1.93	Neutral
15	246	S	S	0	Neutral
16	358	D	G	-5.21	Deleterious
17	644	P	P	0	Neutral
18	376	Y	Y	0	Neutral
19	594	R	R	0	Neutral
20	552	P	P	0	Neutral
21	191	G	G	0	Neutral
22	331	R	W	-3.74	Deleterious
23	5	A	P	-1.25	Neutral
24	453	A	A	0	Neutral
25	440	D	N	-3.08	Deleterious
26	561	K	R	-1.28	Neutral
27	515	A	T	-0.48	Neutral
28	320	E	D	-0.9	Neutral
29	510	V	M	-1.63	Neutral
30	472	A	A	0	Neutral
31	256	P	P	0	Neutral
32	610	N	D	-2.1	Neutral
33	412	N	N	0	Neutral
34	592	N	S	-1.8	Neutral
35	716	G	G	0	Neutral
36	659	P	P	0	Neutral
37	307	Y	Y	0	Neutral
38	31	Q	Q	0	Neutral
39	467	T	T	0	Neutral
40	604	L	L	0	Neutral
41	335	R	W	-4.66	Deleterious
42	443	N	N	0	Neutral
43	196	F	F	0	Neutral
44	358	D	D	0	Neutral
45	612	H	Y	-1.83	Neutral
46	642	R	W	-2.44	Neutral
47	783	L	L	0	Neutral
48	186	G	R	-6.53	Deleterious
49	55	R	W	-5.97	Deleterious
50	754	G	R	-0.17	Neutral
51	391	V	V	0	Neutral
52	172	R	H	-1.5	Neutral
53	674	T	M	-3.4	Deleterious
54	319	R	Q	-0.51	Neutral
55	645	P	P	0	Neutral
56	144	L	L	0	Neutral
57	653	P	T	0.74	Neutral
58	104	P	P	0	Neutral
59	154	P	R	-0.51	Neutral
60	3	G	R	-0.59	Neutral
61	638	P	L	-1.46	Neutral
62	518	L	I	-1.14	Neutral
63	489	A	V	-1.64	Neutral
64	674	T	T	0	Neutral
65	4	P	L	-0.38	Neutral
66	758	A	A	0	Neutral
67	602	L	L	0	Neutral
68	134	V	M	-1.05	Neutral
69	318	D	E	-0.8	Neutral
70	70	L	L	0	Neutral
71	162	A	V	-0.08	Neutral
72	190	E	E	0	Neutral
73	664	R	H	0	Neutral
74	653	P	P	0	Neutral
75	577	E	G	-1.53	Neutral
76	247	E	K	-2.32	Neutral
77	255	D	D	0	Neutral
78	264	S	S	0	Neutral
79	679	T	T	0	Neutral
80	492	R	W	-3.05	Deleterious
81	349	I	I	0	Neutral
82	361	P	P	0	Neutral
83	668	D	E	-0.32	Neutral
84	489	A	A	0	Neutral
85	755	A	A	0	Neutral
86	732	A	V	-0.9	Neutral
87	778	D	D	0	Neutral
88	418	Y	C	-6.53	Deleterious
89	257	E	K	-0.21	Neutral
90	620	T	T	0	Neutral
91	55	R	L	-4.83	Deleterious

Table 3: Disorder effects on nsSNPs associated variations(predicted)showed by SNPs&GO

Mutation	Prediction	RI	Probability

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GABARAP			
R15H	Harmless(neutral)	8	0.101
	Harmless (neutral)	1	0.433
	Harmless (neutral)	9	0.043
V33A	Harmless (neutral)	1	0.433
	Harmless (neutral)	2	0.403
	Harmless (neutral)	6	0.212
H69Y	Disorder	0	0.523
	Harmless (neutral)	3	0.36
	Harmless (neutral)	6	0.212
E73V	Disorder	7	0.831
	Disorder	1	0.567
	Disorder	2	0.584
ZDHHC3			
M1I	Disorder	4	0.3
	Disorder	4	0.291
M1V	Disorder	5	0.271
	Disorder	4	0.303
Y29C	Neutral	3	0.347
	Neutral	7	0.14
I115F	Disorder	5	0.229
	Disorder	2	0.582
	Neutral	9	0.074
V74M	Neutral	4	0.276
	Disease	8	0.894
	Neutral	6	0.215
I213V	Neutral	6	0.184
	Disorder	4	0.699
	Neutral	8	0.089
S240T	Harmless (neutral)	8	0.083
	Disease	5	0.747
	Neutral	8	0.08

Table 4: Stability analysis(predicted) showed by I-Mutant version 3.0

S. NO .	AMINO ACID CHANGE	STABILITY	RI
GABARAP			
1	V33A	DECREASE	9
2	E73V	INCREASE	4
3	R231H	NOT FOUND	
4	R98H	DECREASE	4
5	H69Y	INCREASE	1
6	L243P	NOT FOUND	
7	L110P	INCREASE	2
NLGN2			
1	R331W	NOT SHOWING	
2	R185W	DECREASE	8
3	R189W	DECREASE	8
4	R335W	NOT SHOWING	
5	H612Y	NOT SHOWING	
6	R642W	NOT SHOWING	
7	G186R	DECREASE	6
8	G40R	INCREASE	0
9	T674M	NOT SHOWING	

ZDHHC3			
1	M1V	DECREASE	6
2	M1I	DECREASE	5
3	I14N	DECREASE	8
4	V168G	DECREASE	8
5	R148W	DECREASE	4
6	R14W	DECREASE	7
7	R182W	DECREASE	4
8	I115F	DECREASE	4
9	I149F	DECREASE	5
10	G44A	DECREASE	9

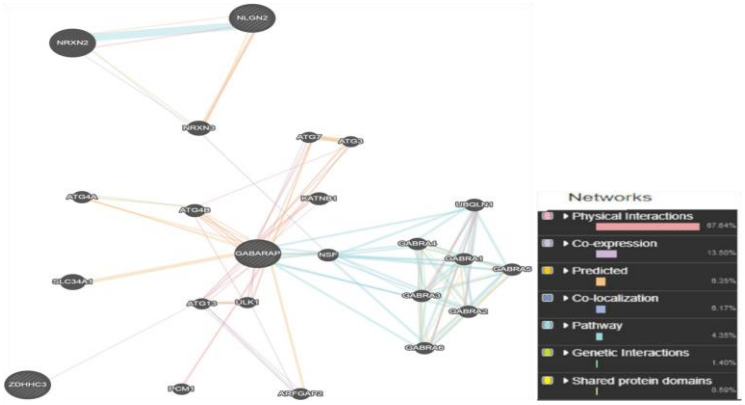

Fig 3: Interaction between GABARAP, NLGN2 and ZDHHC3 genes & related genes by using GeneMANIA

Conclusion

Three deleterious SNPs of GABARAP, one SNP (rs201481724) for NLGN2 and three SNPs for ZDHHC3 are found which affect the function, structure and the stability of gene by various *in silico* bioinformatics (servers) tools. These methods for projection used standard tools and multiple disciplines for detecting and characterizing the missense variation. In order to increase the accuracy and for ensuring our results; four web servers have been used: SIFT, Provean, SNP&GO, and I mutant. These genes were studied because they are related to GABA receptors, one of the main receptor in causing epilepsy; also these genes have much evidence of associated with the disorder.

The current study illustrates that rs2134510, rs367707073, and rs372747001 for GABARAP protein, rs150545990, rs201481724, rs201972977, and rs376668595 for NLGN2 gene and rs140002813, rs143591132 and rs376006143 for ZDHHC3 may have role in the disease. Thus these mutations might be responsible for the pathophysiology of the disease.

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