Comprehensive bioinformatics analysis of *L1CAM* gene revealed Novel Pathological mutations associated with L1 syndrome

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Abstract:

Background: *Mutations in the human L1CAM gene cause a group of neurodevelopmental disorders known as L1 syndrome (CRASH syndrome). The L1CAM gene provides instructions for producing the L1 protein, which is found all over the nervous system on the surface of neurons. L1 syndrome involves a variety of characteristics but the most common characteristic is muscle stiffness. Patients with L1 syndrome can also suffer from difficulty speaking, seizures, and underdeveloped or absent tissue connecting the left and right halves of the brain*. **Method***: The human L1CAM gene was studied from dbSNP/NCBI, 1499 SNPs were Homo sapiens; of which 450 were missense mutations. This selected for Comprehensive bioinformatics analysis by several in silico tools to investigate the effect of SNPs on L1CAM protein's structure and function.* **Results**: *34 missense mutations (26 novel mutations) out of 450 nsSNPs that are found to be the most deleterious that effect on the L1CAM structural and functional level.* **Conclusion:** *Better understanding of L1 syndrome caused by mutations in L1CAM gene was achieved using Comprehensive bioinformatics analysis. These findings describe 35 novel L1 mutations which improve our understanding on genotype-phenotype correlation. And can be used as diagnostic markers for L1 syndrome and besides in cancer diagnosis specifically in breast cancer.*

Keywords: *L1CAM, L1 syndrome (CRASH syndrome), Comprehensive bioinformatics analysis, SNPs, diagnostic markers, breast cancer.*

1. Introduction:

L1 syndrome (also known as CRASH syndrome) is a group of a very rare inherited disorders that primarily affect the nervous system and is characterized by Hydrocephalus with Stenosis of the Aqueduct of Sylvius (HSAS), intellectual disability, corpus callosum hypoplasia (or agenesis), adducted thumbs and spastic paraplegia (1-4). It is a recessive X-linked disorder that is exclusively affects men with incidence of 1/30.000 male births (5), and it is caused by mutations in the *L1CAM* gene (6-19) located near the telomere of the long arm of X chromosome in Xq28 in humans (20-22). Over 200 mutations in L1CAM gene have been reported (3, 17), this gene encodes for the L1 Cell Adhesion Molecule protein, which is a member of the immunoglobulin superfamily. As the name implies; the protein enables the adhesion of neural cells to one another and it is a key regulator of synapse formation, synaptic plasticity and axons and dendrites growth and formation (23-26). It was found that *L1CAM* gene is a major driver for tumor cell invasion and motility (27) therefore fully understanding its function will aid in the diagnosis and treatment of different types of cancers (27-50).

The underline pathogenetic mechanisms by which L1 syndrome happens remains unsolved (6, 25, 51), and the treatment requires shunting of the cerebrospinal fluid as needed (52). We hope for a better understanding of the condition and we believe thorough investigation of the *L1CAM* gene might help in that.

The aim of this study is to identify pathogenic mutations in the coding region of *L1CAM* gene using variant bioinformatics tools, which might then be used as diagnostic markers and may help in the development of new therapeutic strategies using gene therapy and pharmacogenomics. This is the first in silico analysis in the coding region of *L1CAM* gene that prioritized the functional analysis of nsSNPs. The use of variant bioinformatics tools was extremely beneficial due to the elimination of the cost and conformation of the results by the different-parameters-based softwares and It will facilitate the future genetic studies (53).

2. Materials and Methods:

2.1 Data mining:

The data on human *L1CAM* gene was collected from National Center for Biological Information (NCBI) web site (54). (https://www.ncbi.nlm.nih.gov/) and the protein sequence was collected from Uniprot (55) (https://www.uniprot.org/).

2.2 SIFT:

We used SIFT to observe the effect of A.A. substitution on protein function. SIFT predicts damaging SNPs on the basis of the degree of conserved amino acid residues in aligned sequences to the closely related sequences, gathered through PSI-BLAST (56, 57). It's available at (http://sift.jcvi.org/).

2.3 PolyPhen:

We used PolyPhen (version 2) to study the probable impacts of A.A. substitution on structural and functional properties of the protein by considering physical and comparative approaches (58, 59). It is available at (http://genetics.bwh.harvard.edu/pph2/).

2.4 PROVEAN:

PROVEAN is a software tool which predicts whether an amino acid substitution or indel has an impact on the biological function of a protein. It is useful for filtering sequence variants to identify nonsynonymous or indel variants that are predicted to be functionally important (60, 61). It is available at (https://rostlab.org/services/snap2web).

2.5 SNAP2:

SNAP2 is a trained classifier that is based on a machine learning device called "neural network". It distinguishes between disease-associated and neutral variants/non-synonymous SNPs by taking a variety of sequence and variant features into account. It is available at (https://rostlab.org/services/snap2web/).

2.6 SNPs&GO:

SNPs&GO is an accurate method that, starting from a protein sequence, can predict whether a variation is disease related or not by exploiting the corresponding protein functional annotation. (62, 63). It is available at (http://snps.biofold.org/snps-and-go/snps-and-go.html).

2.7 PHD-SNP:

An online Support Vector Machine (SVM) based classifier, is optimized to predict if a given single point protein mutation can be classified as disease-related or as a neutral polymorphism. It is available at: (http://snps.biofold.org/phd-snp/phdsnp.html).

2.8 I-Mutant 3.0:

I-Mutant 3.0 Is a neural network based tool for the routine analysis of protein stability and alterations by taking into account the single-site mutations. The FASTA sequence of protein retrieved from UniProt is used as an input to predict the mutational effect on protein stability (64). It is available at (http://gpcr2.biocomp.unibo.it/cgi/predictors/I-Mutant3.0/I-Mutant3.0.cgi).

2.9 MUpro:

MUpro is a support vector machine-based tool for the prediction of protein stability changes upon nsSNPs. The value of the energy change is predicted, and a confidence score between -1 and 1 for measuring the confidence of the prediction is calculated. A score <0 means the variant decreases the protein stability; conversely, a score >0 means the variant increases the protein stability. It's available at (http://mupro.proteomics.ics.uci.edu/).

2.10 GeneMANIA:

We submitted genes and selected from a list of data sets that they wish to query. GeneMANI approach to know protein function prediction integrate multiple genomics and proteomics data sources to make inferences about the function of unknown proteins (65-67). It is available at (http://www.genemania.org/).

2.11 Identification of Functional SNPs in Conserved Regions by using ConSurf server:

ConSurf web server provides evolutionary conservation profiles for proteins of known structure in the PDB. Amino acid sequences similar to each sequence in the PDB were collected and aligned using CSI-BLAST and MAFFT, respectively. The evolutionary conservation of each amino acid position in the alignment was calculated using the Rate 4Site algorithm, implemented in the ConSurf web server. It is available at (http://consurf.tau.ac.il/).

2.12. Structural Analysis:

2.12.1 Detection of nsSNPs Location in Protein Structure:

Mutation3D is a functional prediction and visualization tool for studying the spatial arrangement of amino acid substitutions on protein models and structures. Further, it presents a systematic analysis of whole genome and whole-exome cancer datasets to demonstrate that mutation3D identifies many known cancer genes as well as previously underexplored target genes (68). It is available at (http://mutation3d.org).

2.13 Analysis of 3 UTR and 5 UTR of L1CAM gene:

Sequence related to the 3 UTR and 5 UTR of *L1CAM* gene were retrieved from ensemble website, (https://www.ensembl.org/index.html) .which were inserted in to RegRNA 2 website to generate the related microRNA sequences. (http://regrna2.mbc.nctu.edu.tw/) These results are also given to miRmap software to get free energy and conservation values. (https://mirmap.ezlab.org/).

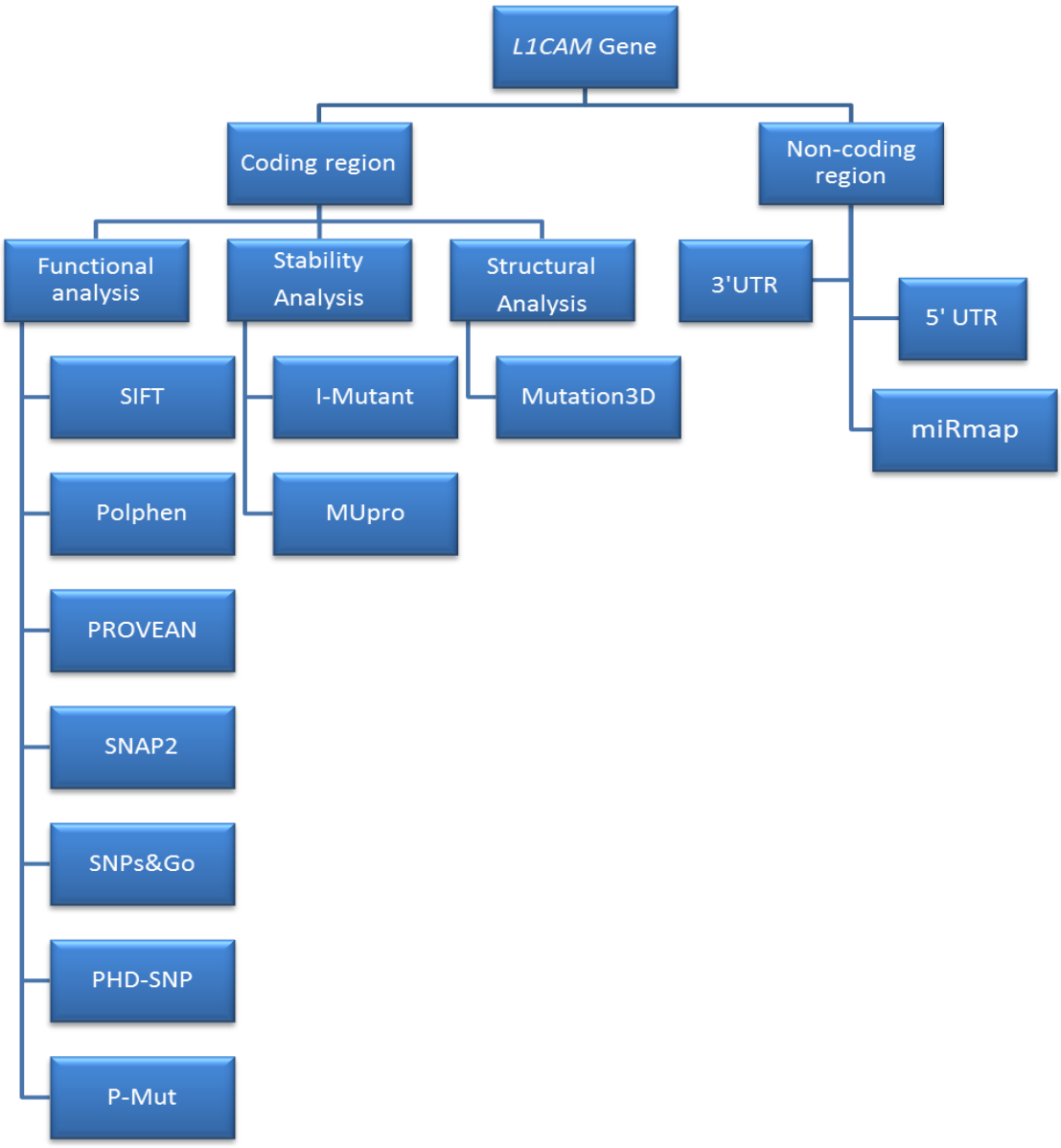

Figure 1: Diagrammatic representation for *L1CAM* gene in coding region in silico work flow.

3. Results:

Table1: Damaging (Deleterious) nsSNPs associated variations predicted by various softwares:

	SIFT		PolyPhen		PROVEAN		SNAP2	
SUB	prediction	Score	prediction	Score	prediction	Score	prediction	Score
E1175K	Affect	0	probably damaging	1	Deleterious	-3.609	Effect	63
K1150R	Affect	0	probably damaging	1	Deleterious	-2.81	Effect	42
G1149R	Affect	0	probably damaging	1	Deleterious	-7.494	Effect	59
R1145H	Affect	0	probably damaging	1	Deleterious	-4.506	Effect	76
R1145C	Affect	0	probably damaging	1	Deleterious	-7.228	Effect	66
L1135V	Affect	0	probably damaging	1	Deleterious	-2.844	Effect	38
L1132P	Affect	0	probably damaging	1	Deleterious	-6.585	Effect	13
R990C	Affect	0	probably damaging	1	Deleterious	-4.943	Effect	41
N945I	Affect	0	probably damaging	1	Deleterious	-8.052	Effect	44
N825K	Affect	0	probably damaging	1	Deleterious	-5.312	Effect	46
N825Y	Affect	0	probably damaging	1	Deleterious	-7.206	Effect	48
P816S	Affect	0	probably damaging	1	Deleterious	-7.806	Effect	43
Y784C	Affect	0	probably damaging	1	Deleterious	-8.014	Effect	65
F781L	Affect	0	probably damaging	1	Deleterious	-5.676	Effect	64
R755H	Affect	0	probably damaging	1	Deleterious	-4.822	Effect	62
R755C	Affect	0	probably damaging	1	Deleterious	-7.697	Effect	62
V752M	Affect	0	probably damaging	1	Deleterious	-2.909	Effect	50
Y750S	Affect	0	probably damaging	1	Deleterious	-8.797	Effect	81
Y682C	Affect	0	probably damaging	1	Deleterious	-7.038	Effect	44
L606F	Affect	0	probably damaging	1	Deleterious	-3.44	Effect	39
D598N	Affect	0	probably damaging	1	Deleterious	-4.922	Effect	62
G587R	Affect	0	probably damaging	1	Deleterious	-7.374	Effect	56
C539G	Affect	0	probably damaging	1	Deleterious	-11.611	Effect	83
G493R	Affect	0	probably damaging	1	Deleterious	-7.078	Effect	79
L482P	Affect	0	probably damaging	1	Deleterious	-6.805	Effect	89
G452R	Affect	0	probably damaging	1	Deleterious	-5.917	Effect	68
F451L	Affect	0	probably damaging	1	Deleterious	-5.834	Effect	69
G411R	Affect	0	probably damaging	1	Deleterious	-7.27	Effect	61
N408K	Affect	0	probably damaging	1	Deleterious	-5.692	Effect	81
G370R	Affect	0	probably damaging	1	Deleterious	-7.853	Effect	50
P346Q	Affect	0	probably damaging	1	Deleterious	-5.699	Effect	17
P333R	Affect	0	probably damaging	1	Deleterious	-8.83	Effect	42
N316S	Affect	0	probably damaging	1	Deleterious	-4.906	Effect	74
G308D	Affect	0	probably damaging	1	Deleterious	-6.868	Effect	60

E304K	Affect	0	probably damaging	1	Deleterious	-2.79	Effect	10
L297V	Affect	0	probably damaging	1	Deleterious	-2.743	Effect	31
W276R	Affect	0	probably damaging	1	Deleterious	-13.728	Effect	71
C264Y	Affect	0	probably damaging	1	Deleterious	-10.779	Effect	78
P240L	Affect	0	probably damaging	1	Deleterious	-9.173	Effect	26
R184Q	Affect	0	probably damaging	1	Deleterious	-3.922	Effect	60
A123T	Affect	0	probably damaging	1	Deleterious	-3.911	Effect	18
A116G	Affect	0	probably damaging	1	Deleterious	-3.868	Effect	62
C57Y	Affect	0	probably damaging	1	Deleterious	-10.604	Effect	79

Table2: Disease effect nsSNPs associated variations predicted by SNPs&GO and PHD-SNP softwares:

		PhD-SNP			SNPs&GO		
dbSNP rs#	SUB	predicton	RI	Score	prediction	RI	Score
rs1064796541	E1175K	Disease	4	0.705	Disease	6	0.822
rs1391080419	K1150R	Disease	2	0.614	Disease	5	0.737
rs200590243	G1149R	Disease	5	0.745	Disease	5	0.745
rs952497509	R1145H	Disease	6	0.799	Disease	4	0.682
rs200798819	R1145C	Disease	8	0.883	Disease	5	0.746
rs1363914541	L1132P	Disease	7	0.838	Disease	2	0.597
rs782573655	R990C	Disease	1	0.561	Disease	3	0.653
rs1375240951	N945I	Disease	5	0.759	Disease	5	0.753
rs1273221058	N825K	Disease	2	0.602	Disease	1	0.551
rs782502555	N825Y	Disease	3	0.67	Disease	3	0.673
rs782776836	P816S	Disease	5	0.744	Disease	4	0.724
rs797045674	Y784C	Disease	7	0.841	Disease	2	0.622
rs948680832	R755C	Disease	3	0.636	Disease	3	0.662
rs1064794855	Y750S	Disease	5	0.768	Disease	3	0.661
rs1412295827	Y682C	Disease	3	0.644	Disease	5	0.755
rs137852519	D598N	Disease	0	0.502	Disease	4	0.676
rs199888009	G587R	Disease	6	0.825	Disease	3	0.644
rs886041102	C539G	Disease	8	0.883	Disease	4	0.69
rs1312463945	G493R	Disease	9	0.931	Disease	4	0.721
rs1064794246	L482P	Disease	9	0.939	Disease	6	0.779
rs137852520	G452R	Disease	9	0.937	Disease	6	0.784
rs1262433336	G411R	Disease	8	0.903	Disease	3	0.661
rs994675918	N408K	Disease	9	0.942	Disease	6	0.791
rs137852524	G370R	Disease	7	0.84	Disease	4	0.691
rs1064793162	P333R	Disease	7	0.871	Disease	3	0.669

rs782052935	N316S	Disease	3	0.653	Disease	3	0.654
rs1322838292	L297V	Disease	5	0.753	Disease	3	0.661
rs1131691900	W276R	Disease	8	0.918	Disease	5	0.731
rs137852518	C264Y	Disease	9	0.949	Disease	6	0.785
rs137852526	P240L	Disease	6	0.788	Disease	2	0.577
rs137852521	R184Q	Disease	6	0.791	Disease	6	0.78
rs782752037	A123T	Disease	4	0.712	Disease	5	0.749
rs782704314	A116G	Disease	8	0.875	Disease	4	0.689
rs1057517755	C57Y	Disease	9	0.933	Disease	7	0.841

***RI: Reliability Index**

Table3: Protein stability analysis predicted by I-Mutant version 3.0 and MUPro:

dbSNP rs#	SUB	SVM2 Prediction Effect	RI	DDG Value Prediction	Mupro Prediction	Mupro Score
rs1064796541	E1175K	Decrease	8	-0.88	Decrease	-1.480268
rs1391080419	K1150R	Decrease	3	-0.35	Decrease	-0.267574
rs200590243	G1149R	Decrease	6	-0.71	Decrease	-1.035834
rs952497509	R1145H	Decrease	9	-1.3	Decrease	-0.84271
rs200798819	R1145C	Decrease	9	-1.07	Decrease	-0.542214
rs1363914541	L1132P	Decrease	2	-0.99	Decrease	-2.127182
rs782573655	R990C	Increase	0	-0.68	Decrease	-0.927319
rs1375240951	N945I	Increase	4	0.84	Decrease	-0.0726
rs1273221058	N825K	Decrease	4	-0.14	Decrease	-0.979333
rs782502555	N825Y	Increase	0	0.16	Decrease	-0.47322
rs782776836	P816S	Decrease	7	-1.36	Decrease	-0.906082
rs797045674	Y784C	Increase	1	-1.09	Decrease	-0.406888
rs948680832	R755C	Decrease	0	-0.43	Decrease	-0.35092
rs1064794855	Y750S	Decrease	4	-1.13	Decrease	-1.433702
rs1412295827	Y682C	Increase	3	-1.06	Decrease	-1.010092
rs137852519	D598N	Decrease	4	-0.82	Decrease	-0.884014
rs199888009	G587R	Decrease	6	-0.59	Decrease	-0.666176
rs886041102	C539G	Decrease	8	-1.46	Decrease	-1.934251
rs1312463945	G493R	Decrease	6	-0.63	Decrease	-1.072532
rs1064794246	L482P	Decrease	8	-2.13	Decrease	-1.123863
rs137852520	G452R	Decrease	3	-0.48	Decrease	-0.39364
rs1262433336	G411R	Decrease	6	-0.4	Decrease	-0.513973
rs994675918	N408K	Decrease	6	-0.27	Decrease	-1.652798
rs137852524	G370R	Decrease	6	-0.45	Decrease	-0.969659
rs1064793162	P333R	Decrease	5	-0.8	Decrease	-0.706657
rs782052935	N316S	Decrease	7	-0.36	Decrease	-1.077409

rs1322838292	L297V	Decrease	8	-1.54	Decrease	-1.14464
rs1131691900	W276R	Decrease	7	-0.87	Decrease	-0.691061
rs137852518	C264Y	Increase	1	0.05	Decrease	-0.882971
rs137852526	P240L	Decrease	3	-0.85	Increase	0.3675391
rs137852521	R184Q	Decrease	8	-0.85	Decrease	-1.084048
rs782752037	A123T	Decrease	7	-0.83	Decrease	-1.052762
rs782704314	A116G	Decrease	9	-1.68	Decrease	-1.39165
rs1057517755	C57Y	Decrease	0	-0.22	Decrease	-0.505714

Table 4: The *L1CAM gene* functions and its appearance in network and genome:

Function	FDR	Genes in network	Genes in genome
basal lamina	0.00455213	3	12
extracellular matrix organization	0.04748433	5	292
extracellular structure organization	0.04748433	5	293
basement membrane	0.04748433	3	33
PcG protein complex	0.04748433	3	39
extracellular matrix structural constituent	0.07853391	3	53
telencephalon development	0.10284851	3	61
extracellular matrix part	0.11929111	3	67
cell-cell adhesion	0.32145746	4	258
proteinaceous extracellular matrix	0.41738063	3	110

***FDR:** false discovery rate is greater than or equal to the probability that this is a false positive.

Table (5) The gene co-expressed, share domain and Interaction with *L1CAM gene* network:

Gene 1	Gene 2	Weight	Network group
RPS6KA1	CNTN2	0.013472	Co-expression
LAMC1	LAMB1	0.011781	Co-expression
DPYSL2	L1CAM	0.010225	Co-expression
LAMC1	LAMB1	0.006291	Co-expression
LAMC1	LAMB1	0.010259	Co-expression
NCAM1	CNTN2	0.003526	Co-expression
NCAN	NCAM2	0.004218	Co-expression
NCAN	RANBP9	0.006789	Co-expression
DPYSL2	ALCAM	0.007145	Co-expression
CSNK2A2	CSNK2A1	0.008062	Co-expression
AP2M1	CSNK2B	0.0056	Co-expression
LAMB1	NCAM2	0.029698	Co-expression

LAMC1	LAMB1	0.006012	Co-expression
DPYSL2	NCAM1	0.007519	Co-expression
CSNK2A1	EPHB2	0.004934	Co-expression
CSNK2A2	CSNK2A1	0.005019	Co-expression
NUMB	ALCAM	0.021748	Co-expression
AP2M1	CSNK2B	0.00784	Co-expression
RPS6KA1	CD24	0.005883	Co-expression
CSNK2A2	CSNK2A1	0.004832	Co-expression
AP2M1	CSNK2B	0.001699	Co-expression
LAMC1	LAMB1	0.013055	Co-expression
DPYSL2	LAMC1	0.007021	Co-expression
CSNK2A2	CSNK2A1	0.010276	Co-expression
CSNK2B	CSNK2A2	0.013334	Co-localization
NCAM1	ALCAM	0.016793	Co-localization
DPYSL2	ALCAM	0.008439	Co-localization
DPYSL2	NCAM1	0.008635	Co-localization
LAMC1	LAMB1	0.015038	Co-localization
DPYSL2	CNTN2	0.004072	Co-localization
DPYSL2	NCAM1	0.005002	Co-localization
DPYSL2	NCAN	0.006302	Co-localization
DPYSL2	AP2M1	0.003664	Co-localization
NCAM2	L1CAM	0.710602	Pathway
CNTN2	L1CAM	0.186909	Pathway
NCAM1	L1CAM	0.157474	Pathway
LAMB1	LAMA1	0.005495	Pathway
LAMC1	LAMA1	0.006524	Pathway
LAMC1	LAMB1	0.006629	Pathway
CNTN2	L1CAM	0.082128	Pathway
RANBP9	L1CAM	0.246827	Pathway
EZR	L1CAM	0.070497	Pathway
SHTN1	L1CAM	0.246827	Pathway
CD24	L1CAM	0.246827	Pathway
EPHB2	L1CAM	0.246827	Pathway
RPS6KA1	L1CAM	0.246827	Pathway
ALCAM	L1CAM	0.246827	Pathway
CSNK2A1	L1CAM	0.246827	Pathway
CSNK2A2	L1CAM	0.246827	Pathway
CSNK2B	L1CAM	0.246827	Pathway
NCAM1	L1CAM	0.024356	Pathway
NCAM1	CNTN2	0.157459	Pathway
NCAN	L1CAM	0.132711	Pathway
NCAN	NCAM1	0.254439	Pathway
NUMB	L1CAM	0.099048	Pathway

LAMA1	L1CAM	0.112598	Pathway
LAMB1	L1CAM	0.112598	Pathway
LAMC1	L1CAM	0.112598	Pathway
DPYSL2	L1CAM	0.099048	Pathway
DPYSL2	NUMB	0.77227	Pathway
LAMB1	LAMA1	0.107346	Pathway
LAMC1	LAMA1	0.090899	Pathway
LAMC1	LAMB1	0.070715	Pathway
CSNK2A2	CSNK2A1	0.244724	Pathway
CSNK2B	CSNK2A1	0.282744	Pathway
CSNK2B	CSNK2A2	0.282744	Pathway
EZR	L1CAM	0.583926	Pathway
AP2M1	L1CAM	0.22872	Pathway
CSNK2B	CSNK2A2	0.585786	Physical Interactions
CSNK2A2	CSNK2A1	0.129744	Physical Interactions
CSNK2B	RPS6KA1	0.256079	Physical Interactions
CSNK2B	CSNK2A2	0.05857	Physical Interactions
CSNK2B	CSNK2A1	0.153861	Physical Interactions
CSNK2B	CSNK2A2	0.404456	Physical Interactions
CSNK2B	CSNK2A1	0.304629	Physical Interactions
CSNK2B	CSNK2A2	0.757546	Physical Interactions
CSNK2A2	CSNK2A1	0.149442	Physical Interactions
CSNK2B	CSNK2A1	0.067753	Physical Interactions
CSNK2A2	CSNK2A1	0.070595	Physical Interactions
CSNK2B	CSNK2A2	0.068903	Physical Interactions
CNTN2	L1CAM	0.125183	Physical Interactions
RANBP9	L1CAM	0.0244	Physical Interactions
EZR	L1CAM	0.029924	Physical Interactions
CSNK2A2	CSNK2A1	0.010519	Physical Interactions
CSNK2B	CSNK2A1	0.004473	Physical Interactions
CSNK2B	CSNK2A2	0.021634	Physical Interactions
NCAN	L1CAM	0.138858	Physical Interactions
NCAN	CNTN2	0.205795	Physical Interactions
NUMB	L1CAM	0.046903	Physical Interactions
NUMB	AP2M1	0.021402	Physical Interactions
LAMB1	LAMA1	0.140696	Physical Interactions
LAMC1	LAMA1	0.129649	Physical Interactions
LAMC1	LAMB1	0.168184	Physical Interactions
DPYSL2	NUMB	0.076553	Physical Interactions
CNTN2	L1CAM	0.312812	Physical Interactions
CSNK2B	CSNK2A1	0.130029	Physical Interactions
CSNK2B	CSNK2A2	0.231819	Physical Interactions
NUMB	AP2M1	0.213141	Physical Interactions

CSNK2B	CSNK2A1	0.707107	Physical Interactions
CSNK2B	CSNK2A2	0.707107	Physical Interactions
CSNK2A2	CSNK2A1	0.258272	Physical Interactions
CSNK2B	CSNK2A1	0.16672	Physical Interactions
CSNK2A2	CSNK2A1	0.023259	Physical Interactions
NUMB	AP2M1	0.160943	Physical Interactions
CSNK2A2	CSNK2A1	0.011126	Physical Interactions
CSNK2B	CSNK2A1	0.001354	Physical Interactions
CSNK2B	CSNK2A2	0.015137	Physical Interactions
CNTN2	L1CAM	0.077965	Physical Interactions
RANBP9	L1CAM	0.031513	Physical Interactions
EZR	L1CAM	0.025337	Physical Interactions
RPS6KA1	L1CAM	0.038565	Physical Interactions
CSNK2A1	L1CAM	0.009078	Physical Interactions
CSNK2B	CSNK2A1	0.003991	Physical Interactions
CSNK2B	CSNK2A2	0.00796	Physical Interactions
NCAM1	L1CAM	0.055163	Physical Interactions
NCAN	L1CAM	0.114873	Physical Interactions
NCAN	CNTN2	0.177063	Physical Interactions
NCAN	NCAM1	0.125278	Physical Interactions
NUMB	L1CAM	0.051243	Physical Interactions
DPYSL2	NUMB	0.116032	Physical Interactions
RPS6KA1	L1CAM	0.043903	Predicted
CSNK2A1	L1CAM	0.020959	Predicted
CSNK2A2	CSNK2A1	0.014636	Predicted
CSNK2B	CSNK2A1	0.011705	Predicted
CSNK2B	CSNK2A2	0.023552	Predicted
NCAN	L1CAM	0.13223	Predicted
NCAN	CNTN2	0.168735	Predicted
NCAN	NCAM1	0.139625	Predicted
NUMB	L1CAM	0.150166	Predicted
DPYSL2	NUMB	0.370674	Predicted
LAMB1	AP2M1	0.040814	Predicted
LAMB1	LAMA1	0.129566	Predicted
EZR	L1CAM	0.707107	Predicted
AP2M1	L1CAM	0.707107	Predicted
LAMC1	LAMA1	0.444927	Predicted
LAMC1	LAMB1	0.205319	Predicted
RANBP9	L1CAM	1	Predicted
CSNK2B	CSNK2A1	0.046556	Predicted
LAMC1	LAMB1	0.696676	Predicted
LAMC1	LAMB1	0.346552	Predicted
CSNK2B	CSNK2A1	1	Predicted

NCAM2	L1CAM	0.016527	Shared protein domains
CNTN2	L1CAM	0.013281	Shared protein domains
CNTN2	NCAM2	0.013968	Shared protein domains
CSNK2A2	CSNK2A1	0.003896	Shared protein domains
NCAM1	L1CAM	0.015744	Shared protein domains
NCAM1	NCAM2	0.031679	Shared protein domains
NCAM1	CNTN2	0.013306	Shared protein domains
LAMB1	LAMA1	0.020749	Shared protein domains
LAMC1	LAMA1	0.036784	Shared protein domains
LAMC1	LAMB1	0.0321	Shared protein domains
NCAM2	L1CAM	0.017744	Shared protein domains
CNTN2	L1CAM	0.01767	Shared protein domains
CNTN2	NCAM2	0.016193	Shared protein domains
CSNK2A1	RPS6KA1	0.002857	Shared protein domains
CSNK2A2	RPS6KA1	0.002857	Shared protein domains
CSNK2A2	CSNK2A1	0.003275	Shared protein domains
NCAM1	L1CAM	0.017358	Shared protein domains
NCAM1	NCAM2	0.015908	Shared protein domains
NCAM1	CNTN2	0.015841	Shared protein domains
LAMB1	LAMA1	0.030715	Shared protein domains
LAMC1	LAMA1	0.044623	Shared protein domains
LAMC1	LAMB1	0.047783	Shared protein domains

Table (6): Common miRNAs identified in 3 UTR region by miRmap software:

MiRNA	Position	No of Nucleotides	Target Sequence	ΔG open	Probability exact	Conservation PhyloP	miRmap score
Has miR-4707-3p	681 ~ 703	23	actacctggctgcggggcgggca	26.84	0.08	0.76	-0.21
hsa-miR-4763-3p	749 ~ 778	30	acccccaggacccctagcagccctgccc	15.73	0.57	0.55	-0.03

Table (7): Common miRNAs identified in 5 UTR region by miRmap software:

miRNA	Position	No of Nucleotides	Target Sequence	ΔG open	Probability exact	Conservation PhyloP	miRmap score
hsa-miR-3189-5p	136 ~ 161	26	ccatgcacaggggagctgatggggca	36.86	0.68	0.57	0
hsa-miR-4743-5p	315 ~ 336	22	cagccccctccccctccggccg	27.09	0.42	0.92	-0.03

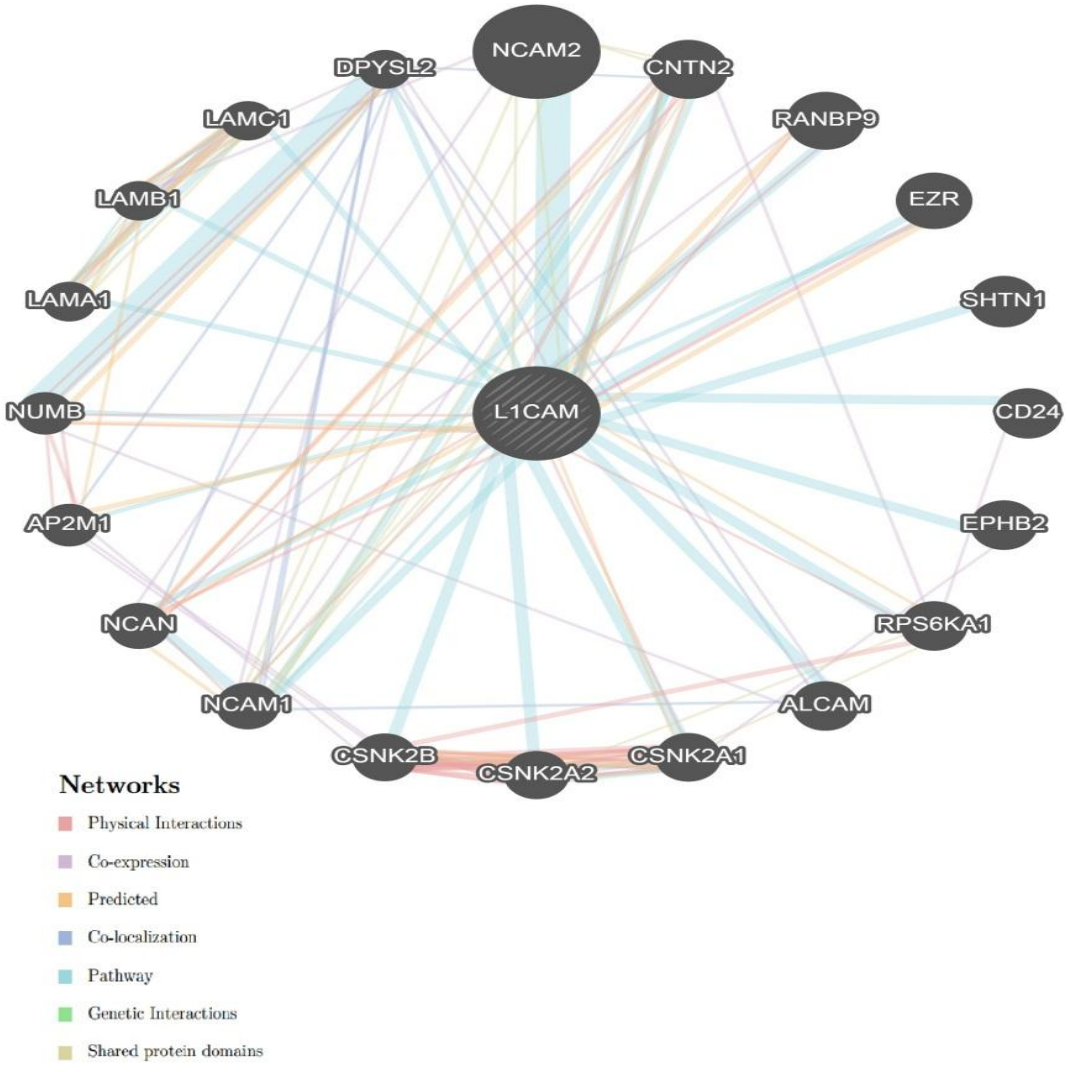

Figure (2): Interaction between *L1CAM* and its related genes.

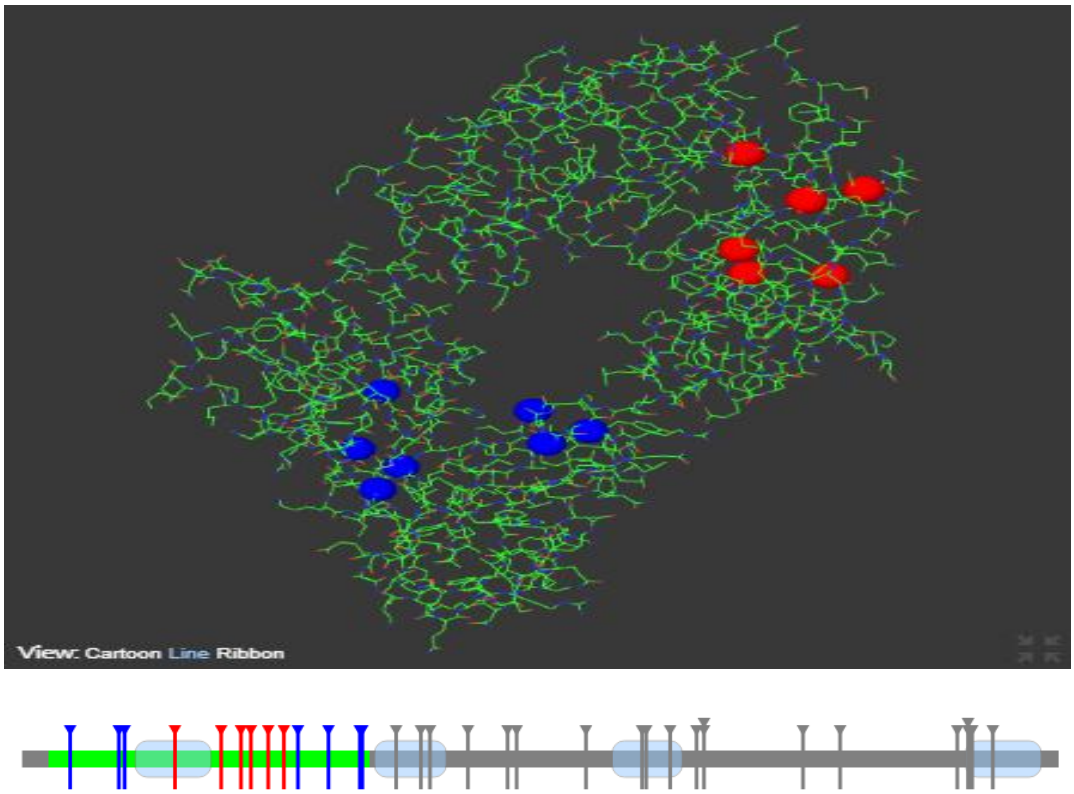

Figure (3): Structural models for wild type *L1CAM,* illustrated by Mutation3D.

4. Discussion:

26 novel mutations were found to have a damaging effect on the stability and function of the *L1CAM* gene using Comprehensive bioinformatics analysis tools. L1 Cell Adhesion Molecule (*L1CAM*) gene is a Protein Coding gene that plays a role In the axon outgrowth and path finding during the development of the nervous system. It is specialized extensions of neurons that transmit nerve impulses. *L1CAM* has been identified in a group of overlapping X-linked neurological disorders known as L1 syndrome (52).

Many *Homo sapiens* SNPs that are now recognized (https://www.ncbi.nlm.nih.gov/snp), open the doors to improve our understanding on genotype-phenotype correlation. Therefore, a deep comprehensive bioinformatics analysis was made to prioritize SNPs that have a structural and functional impact on L1CAMprotein. The most frequent type of genetic mutation is the single nucleotide polymorphism (SNP). Non-synonymous SNPs (nsSNPs) or missense mutations arise in coding region. nsSNPs result in a single amino acid substitution which may have effects on the structure and/or function of protein (69). Therefore, in this study we focus on SNPs in coding and non-coding regions. We investigated the effect of each SNP on the function and stability of the protein and gene expression of related genes using different bioinformatics tools with different parameters and aspects, in order to confirm the results we found and to

minimize the error to the least percentage possible. The software used were SIFT, Polyphen-2, PROVEAN, SNAP2, SNP&GO, PhD-SNP, I-Mutant 3.0, MUPro and Mutation3D (Figure 1)

1498 SNPs were retrieved from the dbSNP/NCBI Database, which was the total number of nsSNPs in the coding region of the *L1CAM* gene. There were 450 nsSNPs (missense mutations) then submitted them to functional analysis by SIFT, PolyPhen-2, PROVEAN and SNAP2. SIFT server predicted 140 deleterious SNPs, polyphen-2 predicted 224 damaging SNPs (75 possibly damaging and 149 probably damaging), PROVEAN predicted 146 deleterious SNPs while in SNAP2 we filtered the triple-positive deleterious SNPs from the previous three analysis tools, out of 49, There were 43 nsSNPs predicted deleterious SNPs by SNAP2. (Table 1) After filtering the Quadra-positive deleterious SNPs we ended up with 43 SNPs and we submitted them to SNPs&GO and PhD-SNP to further investigate their effect on the functional level. PhD-SNP predicted 37 disease-associated SNPs while SNP&GO predicted 38, so we filtered the double positive 34 SNPs (Table 2) and submitted them to I-Mutant 3.0, P-MUT and MUPro respectively (Table 3) to investigate their effect on structural level. All the SNPs were found to cause a decrease in the stability of the protein except for six SNPs predicted by I-Mutant 3.0 to increase the stability, one SNP (P240L) prediction by MUPro and the SNPs in P-MUT predicted 34 deleterious.

Interestingly, GeneMANIA could not predict *L1CAM* gene function after the mutations. The genes co-expressed with, share similar protein domain, or participate to achieve similar function were illustrated by GeneMANIA and shown in (figure 2), Tables (4 & 5).

We also used ConSurf server; the nsSNPs that are located at highly conserved amino acid positions tend to be more deleterious than nsSNPs that are located at non-conserved sites. (supplemental figure 4 for ConSurf result, which is available at https://www.biorxiv.org/)

There were some studies that have been reported which show pathogenic nsSNPs that cause L1 syndrome (11, 70-78), which is corresponding with our results. It has been observed that *L1CAM* polymorphisms are associated in several types of human cancers (27-50, 79). Furthermore, this study confirm that (E1175K, Y784C, Y750S, D598N, C539G, G452R, G370R, P333R, W276R, C264Y, P240L and R184Q) SNPs are pathogenic, these results show similarities with the results found earlier in dbSNPs/NCBI database. Also all these SNPs were retrieved as untested (C57Y, A116G, A123T, L297V, N316S, N408K, G411R, G493R, Y682C, R755C, P816S, N825Y, N825K, N945I, R990C, L1132P, R1145C, R1145H, G1149R, and K1150R) were found to be all pathogenic.

We also used Mutation3D server; (Figure 3) All SNPs in red (R184Q, P240L, C264Y, W276R, L297V and N316S) are clustered mutation. Significantly, such mutation clusters are abundant in human cancers (82) we think it is associated with breast cancer. Afflictions which

can be considered cases of highly accelerated evolution within somatic tissues. Recent studies have revealed several molecular mechanisms of clustered mutagenesis. (83) While SNPs in blue (C57Y, A116G, A123T, P333R, G370R, N408K and G411R) and gray are covered and uncovered mutation respectively.

Different cellular functions are controlled by transcriptional regulation done by non-coding RNA molecules. Recently discovered class of non-coding RNA molecules is MicroRNA (miRNA) that are small non-coding RNA having function of activation and/or suppression of protein translation inside the cells at post-transcriptional level (84). In present study, we have predicted some targets miRNAs for *L1CAM* gene. we found that it has sites for (Has miR-4707-3p) and (hsa-miR-4763-3p) microRNA in the 3 UTR regions. And sites for (hsa-miR-3189-5p) and (hsa-miR-4743-5p) microRNA in the 5 regions. Which were noted to be conserved among different species indicating its significant role in the function of the final protein. This insight provides clue to wet-lab researches to understand the expression pattern of *L1CAM* gene and binding phenomenon of mRNA and miRNA upon mutation.

This study revealed *26 novel* Pathological mutations that have a potential functional impact and may thus be used as diagnostic markers for L1 syndrome and can make an ideal target for tumor therapy (32, 80) properties of *L1CAM*, in addition to its cell surface localization, make it a potentially useful diagnostic marker for cancer progression and a candidate for anti-cancer therapy (39, 81). Finally some appreciations of wet lab techniques are suggested to support these findings.

5. Conclusion:

A large number of different pathological *L1CAM* mutations have been identified. Therefore the confirmation of these nsSNPs in L1 syndrome was crucial by using Comprehensive bioinformatics analysis. These findings describe *26* novel L1 mutations which improve our understanding on genotype-phenotype correlation. And can be used as diagnostic markers for L1 syndrome and besides in cancer diagnosis.

Conflict of interest:

The authors declare no conflict of interest.

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