Insilico Analysis Reveal Three novel nsSNPs May effect on *GM2A* protein Leading to AB variant of GM2 gangliosidosis

Tebyan A. Abdelhameed¹, Mujahed I. Mustafa^{1,2}, Dina N. Abdelrahman^{1,3}, Fatima A. Abdelrhman¹, Mohamed A. Hassan¹.

1. Department of biotechnology, Africa City of Technology-Khartoum, Sudan.

2. Department of Biochemistry, University of Bahri-Khartoum, Sudan.

3. Central laboratory, Ministry of Higher education and scientific research- Khartoum, Sudan.

Corresponding author: Tebyan A. Abdelhameed: tebyanameer2511@gmail.com.

ABSTRACT

Background: AB variant of GM2 gangliosidosis caused as a result of mutations in GM2 activator gene (*GM2A*) which is regarded as neurodegenerative disorder. This study aimed to predict the possible damaging SNPs of this gene and their impact on the protein using different bioinformatics tools. **Methods:** SNPs retrieved from the NCBI database were analyzed using several bioinformatics tools. The different tools collectively predicted the effect of single nucleotide substitution on both structure and function of GM2 activator. **Results:** Three novel mutations were found to be highly damaging to the structure and function of the *GM2A* gene. **Conclusion:** Four SNPs were found to be highly damaging SNPs that affect function, structure and stability of *GM2A* protein, which they are: C99Y, C112F, C138S and C138R, three of them are novel SNPs (C99Y, C112F and C138S). Also 46 SNPs were predicted to affect miRNAs binding sites on 3'UTR leading to abnormal expression of the resulting protein. These SNPs should be considered as important candidates in causing AB variant of GM2 gangliosidosis and may help in diagnosis and genetic screening of the disease.

Keywords: *GM2 gangliosidosis- AB variant, GM2A, neurodegenerative disorder, bioinformatics, single nucleotide polymorphisms (SNPs), Computational, insilico.*

1. INTRODUCTION:

GM2-gangliosidosis is a group of neurological disorders resulting from genetically defective catabolism, and consequent abnormal accumulation, of GM2-ganglioside. Three major types are distinguished: the B variant (Tay-Sachs disease), the O variant (Sandhoff disease), and the AB variant, caused by genetic abnormalities in the genes coding for the beta-hexosaminidase alpha- or beta-subunit, or the GM2-activator protein, respectively. In this study we will focus only in the AB variant of GM2 gangliosidosis.(1, 2) Variant AB of infantile GM2 gangliosidosis is a fatal disease leading invariably to death within the first few years of life, due to the excessive storage of the glycolipids GM2 and GA2 which occurs in the nervous tissue of the patient.(3, 4) The variant AB is characterized by a normal or close to the normal range of this enzyme. Thus, variant AB is an example of a fatal lipid storage disease that is caused not by a defect of a degrading enzyme but rather by a defective factor necessary for the interaction of lipid substrates and the water-soluble hydrolase.(3, 5-7)

The most reported gene for this disorder is *GM2A* (8, 9) which acts as a cofactor for the enzyme. (10, 11) located in chromosome 5q31.3-q33.1 (12-14) The GM2 activator deficiency is caused by mutations in the *GM2A* gene encoding the GM2 activator protein.(15) Different mutations have been reported related to *GM2A*, (16-22) but interestingly, some study shows that, mutation in *GM2A* Leads to a Progressive Chorea-dementia Syndrome.(23)

ELISA system can be used as diagnosis tool as well as therapeutic evaluation of GM2 gangliosidoses using anti-GM2 ganglioside antibodies(24) Can also be diagnosed by demonstrating accumulation of GM2 in the CSF of patients with normal hexosaminidase activity.(25) Biochemical and molecular analysis can also be additional diagnosis approaches.(10, 26-28) Some study revealed that, not all GM2-gangliosidosis pateints can have mutations in the protein coding region of the GM2 activator gene.(29)

The usage of in silico studies has strong impact on the identifcation of candidate SNPs since they are easy and less costly and can facilitate future genetic studies.(30) The main objective of this study was to identify the pathogenic SNPs in coding region and 3'UTR in *GM2A* gene using in silico analysis softwares and to determine the effect of these SNPs on the structural, functional levels, and regulation of their respective proteins.

2. MATERIALS AND METHODS:

2.1 Data mining:

The data on human *GM2A* gene was collected from National Center for Biological Information (NCBI) web site (31) . The SNP information (protein accession number and SNP ID) of the MEFV gene was retrieved from the NCBI dbSNP (<http://www.ncbi.nlm.nih.gov/snp/>) and the protein sequence was collected from Swiss Prot databases (<http://expasy.org/>)(32).

2.2 SIFT:

SIFT is a sequence homology-based tool (33) that sorts intolerant from tolerant amino acid substitutions and predicts whether an amino acid substitution in a protein will have a phenotypic Effect. Considers the position at which the change occurred and the type of amino acid change. Given a protein sequence, SIFT chooses related proteins and obtains an alignment of these proteins with the query. Based on the amino acids appearing at each position in the alignment, SIFT calculates the probability that an amino acid at a position is tolerated conditional on the most frequent amino acid being tolerated. If this normalized value is less than a cutoff, the substitution is predicted to be deleterious. SIFT scores <0.05 are predicted by the algorithm to be intolerant or deleterious amino acid substitutions, whereas scores >0.05 are considered tolerant. It is available at (<http://sift.bii.a-star.edu.sg/>).

2.3 Polyphen-2:

It is a software tool (34) to predict possible impact of an amino acid substitution on both structure and function of a human protein by analysis of multiple sequence alignment and protein 3D structure, in addition it calculates position-specific independent count scores (PSIC) for each of two variants, and then calculates the PSIC scores difference between two variants. The higher a PSIC score difference, the higher the functional impact a particular amino acid substitution is likely to have. Prediction outcomes could be classified as probably damaging, possibly damaging or benign according to the value of PSIC as it ranges from (0_1); values closer to zero considered benign while values closer to 1 considered probably damaging and also it can be indicated by a vertical black marker inside a color gradient bar, where green is benign and red is damaging. nsSNPs that predicted to be intolerant by Sift has been submitted to Polyphen as protein sequence in FASTA format that obtained from UniprotKB /ExPasy after submitting the relevant ensemble protein (ESNP) there, and then we entered position of mutation, native amino acid and the new substituent for both structural and functional predictions. PolyPhen version 2.2.2 is available at (<http://genetics.bwh.harvard.edu/pph2/index.shtml>).

2.4 Provean:

Provean is a software tool (35) 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. It is available at (<https://roslab.org/services/snap2web/>).

2.5 SNAP2:

Functional effects of mutations are predicted with SNAP2 (36). SNAP2 is a trained classifier that is based on a machine learning device called "neural network". It distinguishes between effect and neutral variants/non-synonymous SNPs by taking a variety of sequence and variant features into account. The most important input signal for the prediction is the evolutionary information taken from an automatically generated multiple sequence alignment. Also structural features such as predicted secondary structure and solvent accessibility are considered. If available also annotation (i.e. known functional residues, pattern, regions) of the sequence or close homologs are pulled in. In a cross-validation over 100,000

experimentally annotated variants, SNAP2 reached sustained two-state accuracy (effect/neutral) of 82% (at an AUC of 0.9). In our hands this constitutes an important and significant improvement over other methods. It is available at (<https://roslab.org/services/snap2web/>).

2.6 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://http://snps.biofold.org/phd-snp/phdsnp.html>).

2.7 SNP& 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. SNPs&GO collects in unique framework information derived from protein sequence, evolutionary information, and function as encoded in the Gene Ontology terms, and outperforms other available predictive methods. (37) It is available at (<http://snps.biofold.org/snps-and-go/snps-and-go.html>)

2.8 P-Mut:

PMUT a web-based tool (38) for the annotation of pathological variants on proteins, allows the fast and accurate prediction (approximately 80% success rate in humans) of the pathological character of single point amino acidic mutations based on the use of neural networks. It is available at (<http://mmb.irbbarcelona.org/PMut>).

2.9 I-Mutant 3.0:

I-Mutant 3.0 Is a neural network based tool (39) 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. It is available at (<http://gpcr2.biocomp.unibo.it/cgi/predictors/I-Mutant3.0/I-Mutant3.0.cgi>).

2.10 Project Hope:

Online software is available at: (<http://www.cmbi.ru.nl/hope/method/>). It is a web service where the user can submit a sequence and mutation. The software collects structural information from a series of sources, including calculations on the 3D protein structure, sequence annotations in UniProt and prediction from other software. It combines this information to give analysis for the effect of a certain mutation on the protein structure. HOPE will show the effect of that mutation in such a way that even those without a bioinformatics background can understand it. It allows the user to submit a protein sequence (can be FASTA or not) or an accession code of the protein of interest. In the next step, the user can indicate the mutated residue with a simple mouse click. In the final step, the user can simply click on one of the other 19 amino acid types that will become the mutant residue, and then full report well is generated (40).

2.11 PolymiRTS:

PolymiRTS is a software used to predict 3UTR (un-translated region) polymorphism in microRNAs and their target sites available at (<http://compbio.uthsc.edu/miRSNP/>). It is a database of naturally occurring DNA variations in microRNAs (miRNA) seed region and miRNA target sites. MicroRNAs pair to the transcript of protein coding genes and cause translational repression or mRNA destabilization. SNPs in microRNA and their target sites may affect miRNA-mRNA interaction, causing an effect on miRNA-mediated gene repression. PolymiRTS database was created by scanning 3UTRs of mRNAs in human and mouse for SNPs in miRNA target sites. Then, the effect of polymorphism on gene expression and phenotypes are identified and then linked in the database. The PolymiRTS data base also includes polymorphism in target sites that have been supported by a variety of experimental methods and polymorphism in miRNA seed regions. (42)

2.12 UCSF Chimera (University of California at San Francisco):

UCSF Chimera (<https://www.cgl.ucsf.edu/chimera/>) is a highly extensible program for interactive visualization and analysis of molecular structures and related data, including density maps, supramolecular assemblies, sequence alignments, docking results, trajectories, and conformational ensembles. High-quality images and animations can be generated. Chimera includes complete documentation and several tutorials. Chimera is developed by the Resource for Biocomputing, Visualization, and Informatics (RBVI), supported by the National Institutes of Health (P41-GM103311).(41)

2.13 Raptor X:

RaptorX (<http://raptorx.uchicago.edu/>): It is a web server predicting structure property of a protein sequence without using any templates. It outperforms other servers, especially for proteins without close homologs in PDB or with very sparse sequence profile. The server predicts tertiary structure (43)

2.14 GeneMANIA:

It is gene interaction software that finds other genes which is related to a set of input genes using a very large set of functional association data. Association data include protein and genetic interactions, pathways, co-expression, co-localization and protein domain similarity. GeneMANIA also used to find new members of a pathway or complex, find additional genes you may have missed in your screen or find new genes with a specific function, such as protein kinases. available at (<https://genemania.org/>) (44).

3. RESULTS:

3.1 Retrieval of SNPs from the Database:

All SNPs data related to *GM2A* gene was gathered from dbSNP in National Center of Biotechnology Information (NCBI) database. This gene containing a total of 247 SNPs in coding region, of which 159 were missense, 74 synonymous, 5 nonsense, 8 frame shift, and 676 in 3'untranslated region (3' UTR).

Figure (1): Flowchart of the software used in the analysis processes.

3.2 The functional effect of Deleterious and damaging nsSNPs of *GM2A* by SIFT, Provean PolyPhen-2, and SNAP2

Table (1): Prediction of functional effect of Deleterious and damaging nsSNPs by SIFT, Polyphen-2, PROVEAN and SNAP2.

Variant	Polyphen2 Prediction	Polyphen2 score	PROVEAN score	PROVEAN Prediction (cutoff= -2.5)	SNAP2 Prediction	SNAP2 score
C99Y	possibly damaging	1	-10.833	Deleterious	effect	85
C112F	probably damaging	1	-10.8	Deleterious	effect	81
C138R	probably damaging	1	-12	Deleterious	effect	89
C138S	probably damaging	1	-10	Deleterious	effect	84

3.3 Functional analysis of ADAMTS13 gene using Disease Related and pathological effect of nsSNPs by PhD-SNP, SNPs & GO and PMut softwares:

Table (2): Prediction of disease related nsSNPs through using: PhD-SNP, SNPs & GO and PMut softwares.

Mutation	SNP Prediction	R I	Probability	PhD Prediction	R I	Probability	Pmut Position	Mutation	Prediction
C99Y	Disease	7	0.831	Disease	9	0.949	99	C → Y (Cys → Tyr)	0.82 (90%) Disease
C112F	Disease	3	0.67	Disease	8	0.887	112	C → F (Cys → Phe)	0.82 (90%) Disease
C138R	Disease	3	0.67	Disease	8	0.896	138	C → R (Cys → Arg)	0.82 (90%) Disease
C138S	Disease	2	0.623	Disease	7	0.843	138	C → S (Cys → Ser)	0.82 (90%) Disease

3.4 Prediction of Change in Stability due to Mutation Using I-Mutant 3.0 Server

Table (3): Effect of nsSNPs on protein stability using I-Mutant

dbSNP rs#	Mutation	Prediction	DDG value (score)
rs751417546	C99Y	Increase Protein Stability	-0.05
rs773743799	C112F	Decrease Protein Stability	0.03
rs137852797	C138R	Decrease Protein Stability	-0.24
rs1174735558	C138S	Decrease Protein Stability	-0.82

3.5 Modeling of amino acid substitution effects on protein structure using Chimera and Project Hope Softwares.

Figure (2): Effect of C99Y (rs751417546) SNP on protein structure in which Cysteine mutated into Tyrosine at position 99 using Project HOPE and Chimera..

Figure (3): Effect of C112F (rs773743799) SNP on protein structure in which Cysteine mutated into Phenylalanine at position 112 using project HOPE and Chimera.

Figure (4) Effect of C138R (rs137852797) SNP on protein structure in which Cysteine mutated into Arginine at position 138 using project HOPE and Chimera.

Figure (5) Effect of C138S (rs137852797) SNP on protein structure in which Cysteine mutated into Serine at position 138 using project HOPE and Chimera.

3.6 SNPs effect on 3'UTR Region (miRNA binding sites) in *GM2A* using PolymiRTS Database

Table (4): prediction of SNPs sites in *GM2A* gene at the 3'UTR Region using PolymiRTS

Location	dbSNP ID	Variant type	Wobble base pair	Ancestral Allele	Allele	miR ID	Conservation	miRSite	Function Class	Experiment Support	context + score change
150647156	rs9324686	SNP	N	T	T	hsa-miR-3662	6	aaTCATTTTgta c	D	N	-0.077
						hsa-miR-4698	7	aatCATTTTGta c	D	N	-0.078
						hsa-miR-580-5p	7	AATCATTTtgta c	D	N	-0.112
150647204	rs147601669	SNP	Y	G	G	hsa-miR-4287	4	ctAAGGGAGA atg	D	N	-0.324
						hsa-miR-4469	7	ctaAGGGAGA atg	D	N	-0.136
						hsa-miR-4685-3p	4	ctAAGGGAGA atg	D	N	-0.313
						hsa-miR-4713-5p	10	ctaaGGGAGA Atg	D	N	-0.151

						hsa-miR-629-3p	10	ctaaGGGAGA Atg	D	N	-0.177
						hsa-miR-6867-3p	7	ctaAGGGAGA Atg	D	N	-0.343
						hsa-miR-7113-3p	7	ctaAGGGAGA atg	D	N	-0.123
					A	hsa-miR-6875-3p	10	ctaaGGAAGA Atg	C	N	-0.123
15064724 3	rs148662 528	SNP	Y	G		hsa-miR-4530	4	gcatCTGCTGG gc	D	N	-0.149
					G	hsa-miR-922	4	gcaTCTGCTGg gc	D	N	-0.094
					A	hsa-miR-936	4	gcaTCTACTGg gc	C	N	-0.088
15064726 7	rs750261 89	SNP	N	C		hsa-miR-1255a	2	CTCATCCcggtt a	D	N	-0.149
						hsa-miR-1255b-5p	2	CTCATCCcggtt a	D	N	-0.158
					C	hsa-miR-5187-5p	2	cTCATCCCcggtt a	D	N	-0.149
						hsa-miR-143-3p	2	cTCATCTCcggtt a	C	N	-0.092
						hsa-miR-4770	2	cTCATCTCcggtt a	C	N	-0.092
					T	hsa-miR-6088	2	cTCATCTCcggtt a	C	N	-0.092
15064736 8	rs189626 755	SNP	N	C	C	hsa-miR-4311	2	ctcTCTCTTTca c	D	N	-0.083
					A	hsa-miR-	2	cTCTCTATTtca	C	N	-0.171

					3976		c				
15064737 3	rs104872 4	SNP	N	A							
					T	hsa-miR-4311	2	tctttcTCTCTTT	C	N	-0.115
					C						
15064737 6	rs201142 293	INDEL	N	-		hsa-miR-548az-5p	2	ttCACTTTTttt	O	N	-0.097
					-	hsa-miR-548t-5p	2	ttCACTTTTttt	O	N	-0.097
15064738 8	rs781088 78	SNP	N	T		hsa-miR-3613-3p	7	tTTTTTTGtcact	D	N	-0.019
					T	hsa-miR-4504	7	ttttTTGTCACt	D	N	-0.209
15064741 3	rs116809 628	SNP	Y	G	G	hsa-miR-1304-3p	4	aAGTGAGAgta tt	D	N	-0.064
15064742 2	rs181890 739	SNP	N	C	C	hsa-miR-424-3p	2	tattaACGTTTTt	D	N	-0.133
15064754 8	rs151210 034	SNP	N	G	G	hsa-miR-3130-3p	2	tactagGGTGCA G	D	N	-0.162
						hsa-miR-3923	2	TACTAGTgtgc ag	C	N	-0.168
						hsa-miR-4704-5p	2	taCTAGTGTgc ag	C	N	-0.147
					T	hsa-miR-	2	tactAGTGTGC	C	N	-0.272

						8064		Ag			
15064764 1	rs184763 035	SNP	N	C	C	hsa-miR-4734	3	ggtaCCCGCAGgg	D	N	-0.229
					T	hsa-miR-1205	3	ggtaCCTGCAGgg	C	N	-0.137
15064769 9	rs143552 628	SNP	N	C		hsa-miR-1254	5	ttgctTCCAGGCt	D	N	-0.196
						hsa-miR-3116	5	ttgctTCCAGGCt	D	N	-0.196
						hsa-miR-6079	4	ttGCTTCCAggct	D	N	-0.125
						hsa-miR-671-5p	4	ttGCTTCCAggct	D	N	-0.079
					C	hsa-miR-6828-5p	4	tTGCTTCCAggct	D	N	-0.208
15064773 6	rs112641 014	SNP	N	G	G	hsa-miR-3613-3p	2	TTTTTTGAgacag	D	N	0.061
					C	hsa-miR-3120-5p	2	tttttCAGACAG	C	N	-0.159
15064780 2	rs181571 430	SNP	N	C		hsa-miR-25-5p	2	aacCTCCGCCtcc	D	N	-0.165
						hsa-miR-6087	2	aacctCCGCCTCc	D	N	-0.165
					C	hsa-miR-658	2	aaCCTCCGCctcc	D	N	-0.222
					T	hsa-miR-1827	2	aacctCTGCCTCc	C	N	-0.091

					hsa-miR-1910-3p	2	aaccTCTGCCTcc	C	N	-0.006
					hsa-miR-2467-3p	2	aaCCTCTGCctcc	C	N	-0.124
					hsa-miR-3612	2	aacctcTGCCTCC	C	N	-0.075
					hsa-miR-4257	2	aACCTCTGcctcc	C	N	-0.056
					hsa-miR-650	2	aacctcTGCCTCC	C	N	-0.075
					hsa-miR-6511a-5p	2	aaccTCTGCCTcc	C	N	-0.006
15064783 6	rs186626 408	SNP	N	C	hsa-miR-1273h-5p	2	cctcagCCTCCC A	D	N	-0.146
					hsa-miR-149-3p	2	cctcagCCTCCC A	D	N	-0.068
					hsa-miR-30b-3p	2	cctcagCCTCCC A	D	N	-0.117
					hsa-miR-3689a-3p	2	cctcagCCTCCC A	D	N	-0.124
					hsa-miR-3689b-3p	2	cctcagCCTCCC A	D	N	-0.124
					hsa-miR-3689c	2	cctcagCCTCCC A	D	N	-0.124
					hsa-miR-3929	2	ccTCAGCCTcca	D	N	0.003
					hsa-miR-4419b	2	ccTCAGCCTcca	D	N	-0.006
					hsa-miR-	2	ccTCAGCCTcca	D	N	-0.034

					4478		ca			
					hsa-miR-4728-5p	2	cctcagCCTCCC A	D	N	-0.085
					hsa-miR-485-5p	2	cctCAGCCTCc ca	D	N	-0.066
					hsa-miR-6779-5p	2	cctcagCCTCCC A	D	N	-0.124
					hsa-miR-6780a-5p	2	cctcagCCTCCC A	D	N	-0.138
					hsa-miR-6785-5p	2	cctcagCCTCCC A	D	N	-0.058
					hsa-miR-6799-5p	2	cctcagCCTCCC A	D	N	-0.088
					hsa-miR-6883-5p	2	cctcagCCTCCC A	D	N	-0.058
					hsa-miR-6884-5p	2	cctCAGCCTCc ca	D	N	-0.066
					hsa-miR-7106-5p	2	cctcagCCTCCC A	D	N	-0.065
					hsa-miR-7160-5p	2	CCTCAGCctcc ca	D	N	-0.039
					hsa-miR-4294	2	cctcAGACTCC ca	C	N	-0.1
					hsa-miR-4649-3p	2	CCTCAGActec ca	C	N	-0.11
					A hsa-miR-7162-3p	2	CCTCAGActec ca	C	N	-0.029
15064802	rs355680	INDE	N	-	T hsa-miR-	2	ttttccTTTTTGA	O	N	0.129

2	32	L				3613-3p					
						hsa-miR-651-3p	2	tTTTCCTTttga	O	N	-0.005
					-						
150648025	rs15860	SNP	N	T	T	hsa-miR-3613-3p	2	tccTTTTTGAtt a	D	N	0.129
						hsa-miR-129-5p	2	tgtctCAAAAA Aa	O	N	0.003
						hsa-miR-3672	2	tGTCTCAaaaa aa	O	N	0.001
						hsa-miR-4524a-3p	2	TGTCTCAaaaa aa	O	N	0.004
						hsa-miR-6864-3p	2	tGTCTCAaaaa aa	O	N	0.001
150648243	rs35654352	INDEL	N	-	-	hsa-miR-8055	2	tgtCTCAAAAA aa	O	N	0.002
					A						
						hsa-miR-1290	3	ccaAAAATCC Aga	D	N	0.069
						hsa-miR-3167	7	ccaaAAATCCA ga	D	N	0.054
						hsa-miR-378j	7	ccaaaATCCA GA	D	N	-0.09
150648283	rs200856540	SNP	N	A	A	hsa-miR-4782-5p	7	ccaaaaATCCA GA	D	N	-0.076

					T	hsa-miR-5706	7	ccaaaaATCCA GA	D	N	-0.113
						hsa-miR-6839-5p	7	ccaaaATCCA GA	D	N	-0.048
						hsa-miR-876-5p	7	ccaaAAATCCA ga	D	N	0.054
						hsa-miR-1299	7	ccaaaaTTCCA GA	C	N	-0.081
						hsa-miR-6128	7	ccaaaATTCCA GA	C	N	-0.052
						hsa-miR-875-3p	7	ccaaaaTTCCA GA	C	N	No Change
15064828 4	rs146686 627	INDE L	N	-	C	hsa-miR-1290	3	caAAAATCCA gat	O	N	0.018
						hsa-miR-3167	7	caaAAATCCA gat	O	N	0.054
						hsa-miR-378j	7	caaaAATCCAG At	O	N	-0.09
						hsa-miR-4782-5p	7	caaaaATCCAG At	O	N	-0.076
						hsa-miR-5706	7	caaaaATCCAG At	O	N	-0.113
						hsa-miR-6839-5p	7	caaaAATCCAG At	O	N	-0.048
						hsa-miR-876-5p	7	caaAAATCCA gat	O	N	0.054
						hsa-miR-204-3p	4	caaaaaTCCCA GAt	O	N	-0.062

					hsa-miR-3192-5p	4	caaaaaTCCCA GAt	O	N	-0.057
					hsa-miR-4314	4	caaaaaTCCCA GAt	O	N	-0.138
					hsa-miR-4646-5p	4	caaaaaTCCCA GAt	O	N	-0.069
					hsa-miR-5089-5p	7	caaaAATCCCA gat	O	N	-0.034
					hsa-miR-619-5p	4	caaaaATCCCA GAt	O	N	-0.151
					hsa-miR-6506-5p	4	caaaaATCCCA GAt	O	N	-0.13
15064828 4	rs201799 049	SNP	N	T	hsa-miR-1290	3	caAAAAATCCA gat	D	N	0.069
					hsa-miR-3167	7	caaAAATCCA gat	D	N	0.054
					hsa-miR-378j	7	caaaAATCCAG At	D	N	-0.09
					hsa-miR-4782-5p	7	caaaaATCCAG At	D	N	-0.076
					hsa-miR-5706	7	caaaaATCCAG At	D	N	-0.113
					hsa-miR-6839-5p	7	caaaAATCCAG At	D	N	-0.048
					T hsa-miR-876-5p	7	caaAAATCCA gat	D	N	0.054
				C	hsa-miR-1298-3p	4	caaaaaCCCAG AT	C	N	-0.14

					hsa-miR-629-5p	7	caaAAACCCA gat	C	N	-0.005
					hsa-miR-6776-5p	7	caaaaACCCAG At	C	N	-0.064
					hsa-miR-6839-3p	7	caaAAACCCA gat	C	N	-0.08
					hsa-miR-6861-5p	7	caaaaACCCAG At	C	N	-0.089
15064828 5	rs355501 13	INDE L	N	-	hsa-miR-1290	3	aAAAATCCAg att	O	N	0.018
					hsa-miR-3167	7	aaAAATCCAg att	O	N	0.054
					hsa-miR-378j	7	aaaAATCCAG Att	O	N	-0.09
					hsa-miR-4782-5p	7	aaaaATCCAGA tt	O	N	-0.076
					hsa-miR-5706	7	aaaaATCCAGA tt	O	N	-0.113
					hsa-miR-6839-5p	7	aaaAATCCAG Att	O	N	-0.048
					hsa-miR-876-5p	7	aaAAATCCAg att	O	N	0.054
					hsa-miR-1298-3p	4	aaaaatCCCAG ATt	O	N	-0.145
					hsa-miR-204-3p	4	aaaaaTCCCAG Att	O	N	-0.062
					hsa-miR-3192-5p	4	aaaaaTCCCAG Att	O	N	-0.057

					hsa-miR-4314	4	aaaaaT C CCAG Att	O	N	-0.138
					hsa-miR-4646-5p	4	aaaaaT C CCAG Att	O	N	-0.069
					hsa-miR-5089-5p	7	aaaAAT C CCAg att	O	N	-0.034
					hsa-miR-619-5p	4	aaaaAT C CCAG Att	O	N	-0.151
					hsa-miR-6506-5p	4	aaaaAT C CCAG Att	O	N	-0.13
15064841 4	rs146782 771	SNP	N	G	hsa-miR-4251	2	aTCTCA G Accg tg	D	N	0.09
					hsa-miR-4329	2	aTCTCA G Accg tg	D	N	0.071
					G hsa-miR-6761-5p	2	aTCTCA G Accg tg	D	N	0.084
15064842 2	rs181014 044	SNP	N	A	hsa-miR-3177-3p	3	CCGTGC A tgcc tt	D	N	-0.085
					A hsa-miR-3910	5	ccgtgc A TGCCT T	D	N	0.116
					hsa-miR-3622a-5p	3	cCGTG C Tgcc tt	C	N	-0.022
					hsa-miR-3714	5	ccgtgc C TGCCT T	C	N	0.092
					hsa-miR-4269	3	ccGTGC C TGcc tt	C	N	0.04
					C hsa-miR-564	3	CCGTGC C tgcc	C	N	-0.121

						hsa-miR-6715b-5p	3	tt ccGTGCCTGcc tt	C	N	0.04
						hsa-miR-6808-5p	3	ccgtgCCTGCC Tt	C	N	0.028
						hsa-miR-6893-5p	3	ccgtgCCTGCC Tt	C	N	0.009
						hsa-miR-940	3	ccgtgCCTGCC Tt	C	N	0.037
15064845 7	rs153450	SNP	Y	G	A	hsa-miR-539-5p	3	ggcaccATTCT C	C	N	0.23
						hsa-miR-1302	2	tgtccgGTCCCA A	D	N	-0.001
						hsa-miR-3122	2	tgtccgGTCCCA A	D	N	0.076
						hsa-miR-3649	2	tgtccGTCCCC Aa	D	N	-0.051
						hsa-miR-3913-5p	2	tgtccgGTCCCA A	D	N	0.104
					G	hsa-miR-4298	2	tgtccgGTCCCA A	D	N	0.013
						hsa-miR-1292-5p	2	tgtcCGTTCCC Aa	C	N	-0.051
						hsa-miR-4471	2	tgtccGTTCCCA a	C	N	0.012
15064849 3	rs11262	SNP	N	G	T	hsa-miR-450a-1-3p	2	tgtccgTTCCCA A	C	N	0.161

					hsa-miR-8059	2	tgtccGTTCCCAa	C	N	0.002
150648574	rs185065661	SNP	N	C	hsa-miR-1827	2	ggctgcCTGCCTC	D	N	-0.024
					hsa-miR-4514	7	ggCTGCCTGcctc	D	N	0.059
					C hsa-miR-4692	7	ggCTGCCTGcctc	D	N	0.059
150648707	rs115956086	SNP	N	C	hsa-miR-1293	2	tACCACCCtggtta	D	N	-0.127
					C hsa-miR-4483	2	tACCACCCtggtta	D	N	-0.09
					hsa-miR-132-3p	5	taccacACTGTTA	C	N	0.044
					hsa-miR-212-3p	5	taccacACTGTTA	C	N	0.041
					hsa-miR-4302	2	tacCACACTGtta	C	N	-0.047
					A hsa-miR-4536-5p	3	TACCACActggtta	C	N	-0.077
150648784	rs140540848	SNP	N	A	A hsa-miR-137	10	GCAATAAatcttt	D	N	0.003
150648841	rs3806952	SNP	N	C	hsa-miR-1273f	2	tgatcaCATCTCA	D	N	0.024
					C hsa-miR-143-3p	2	tgatcaCATCTCA	D	N	0.021

						hsa-miR-4708-5p	2	tgatcaCATCTC A	D	N	0.016
						hsa-miR-4770	2	tgatcaCATCTC A	D	N	0.021
						hsa-miR-576-3p	6	tgatCACATCTC a	D	N	-0.049
						hsa-miR-6088	2	tgatcaCATCTC A	D	N	0.035
15064887 8	rs380695 3	SNP	N	C							
						hsa-miR-1225-3p	2	GGGGCTCttt g	D	N	-0.181
					C	hsa-miR-1276	2	gggGCTCTT gg	D	N	0.084
15064900 6	rs125163 91	SNP	Y	A	T	hsa-miR-3613-3p	2	ggggcTTTT Gg	C	N	0.2
						hsa-miR-1468-3p	6	tttgctATTTTGC	D	N	-0.017
					A	hsa-miR-548p	2	TTTGCTAtttgc	D	N	-0.017
						hsa-miR-3120-3p	2	tTTGCTGTtttgc	C	N	-0.044
15064915 3	rs153449	SNP	N	C	G	hsa-miR-545-3p	2	TTTGCTGtttgc	C	N	0.013
						hsa-miR-19a-5p	2	GCAAAACgcct ta	D	N	0.003
						hsa-miR-19b-1-5p	2	GCAAAACgcct ta	D	N	-0.007
					C	hsa-miR-19b-2-5p	2	GCAAAACgcct ta	D	N	-0.007

15064944 2	rs373404 1	SNP	N	T	T	hsa-miR-4709-5p	2	tACTGTTGttgg t	D	N	-0.152
					C	hsa-miR-4645-3p	2	TACTGTCgttg gt	C	N	-0.172
15064953 6	rs149180 405	SNP	N	C		hsa-miR-5584-5p	3	cgTTTCCTctg t	D	N	-0.012
					C	hsa-miR-6873-5p	2	cgttTCCTCTg t	D	N	-0.156
15064959 6	rs113128 701	SNP	Y	G	A	hsa-miR-5002-5p	2	ttgACCAATct c	C	N	-0.07
15064970 0	rs344928 98	INDEL	N	-		hsa-miR-4699-5p	2	ggcATCTCAa tc	O	N	-0.044
					-	hsa-miR-4709-3p	2	ggcaTCCTCAA tc	O	N	-0.025
					T						
15064971 1	rs183242 789	SNP	Y	G		hsa-miR-192-5p	2	tcgcAGGTCAA ag	D	N	-0.155
						hsa-miR-215-5p	2	tcgcAGGTCAA ag	D	N	-0.155
						hsa-miR-3661	2	tcgCAGGTCAA ag	D	N	-0.158
					G	hsa-miR-631	2	tcgCAGGTCAA ag	D	N	-0.158
					A	hsa-miR-7641	2	tcgcAGATCAA ag	C	N	-0.076

15064972 5	rs201627 590	INDEL	N	-	TAA	hsa-miR-4477a	4	gcagactTTAATAAatg	O	N	0.056
					-						
15064972 6	rs143203 938	INDEL	N	-	AAT A	hsa-miR-4477a	4	gcagactTTAATAAatggt	O	N	0.056
					-	hsa-miR-6512-5p	4	gcagactTTAATGGT	O	N	-0.114
15064972 9	rs353846 04	INDEL	N	-		hsa-miR-4477a	4	gactTTAATAAatggtgct	O	N	0.056
					AAA T	hsa-miR-4729	4	gactttaATAAA TGgtgct	O	N	-0.005
						hsa-miR-29a-3p	3	gactttaaTGGTGCT	O	N	0.014
						hsa-miR-29b-3p	3	gactttaaTGGTGCT	O	N	0.014
						hsa-miR-29c-3p	3	gactttaaTGGTGCT	O	N	0.014
						hsa-miR-6512-5p	4	gactTAATGGTgct	O	N	-0.114
					-	hsa-miR-767-5p	4	gactttaATGGTGCt	O	N	-0.002
						hsa-miR-4477a	4	tTTAATAAatggt	D	N	0.056
15064972 9	rs200150 752	SNP	Y	A	A	hsa-miR-4729	4	tttaATAAATGgt	D	N	-0.005
					G	hsa-miR-664a-3p	4	tttAATGAATggt	C	N	0.001

15064981 3	rs111935 946	SNP	Y	A	A	hsa-miR-4653-3p	4	AACTCC A tctgga	D	N	-0.156
15064986 1	rs100760 53	SNP	Y	G		hsa-miR-1237-3p	2	aataCAG A AGGAa	D	N	-0.36
						hsa-miR-1248	2	aatacAG A AGGAa	D	N	-0.134
						hsa-miR-3182	2	aatACAG A AGgaa	D	N	-0.198
						hsa-miR-3686	2	aaTACAG A Aggaa	D	N	-0.082
						hsa-miR-4742-3p	2	AATACAG A aggaa	D	N	-0.273
					G	hsa-miR-6868-3p	2	aatacAG A AGGAA	D	N	-0.294
15064990 8	rs143342 609	SNP	N	T	A	hsa-miR-3149	2	aATACA A Aaggaa	C	N	-0.053
					T	hsa-miR-105-5p	3	gacaCAT T TTGAct	D	N	-0.043
						hsa-miR-7853-5p	3	gacaCAT T TTGAct	D	N	-0.043
						hsa-miR-595	2	gaCACAC T Tga	C	N	-0.215
					C	hsa-miR-6513-3p	3	gacACAC T TGAct	C	N	-0.299

Interactions of *GM2A* gene with other Functional Genes illustrated by GeneMANIA

Figure 3: *GM2A* Gene Interactions and network predicted by GeneMania.

Table (5): The *GM2A* gene functions and its appearance in network and genome

Function	FDR	Genes in network	Genes in genome
glycolipid metabolic process	0.000729126	5	91
membrane lipid metabolic process	0.002107403	5	140
glycosphingolipid metabolic process	0.002107403	4	55
sphingolipid metabolic process	0.018998673	4	102
regulation of hormone levels	0.566018537	4	269
vacuolar part	0.566018537	4	262

Table (6): The genes co-expressed, co localized, Physical Interacted and share a domain with IL2RG

Gene 1	Gene 2	Weight	Network group	Network
<i>GAA</i>	<i>NPC2</i>	0.013373704	Co-expression	Ramaswamy-Golub-2001
<i>GAA</i>	<i>GRN</i>	0.009285928	Co-expression	Ramaswamy-Golub-2001
<i>NPC2</i>	<i>LY86</i>	0.006769352	Co-expression	Wang-Maris-2006
<i>LY96</i>	<i>LY86</i>	0.006558455	Co-expression	Wang-Maris-2006
<i>LY96</i>	<i>NPC2</i>	0.004556628	Co-expression	Wang-Maris-2006
<i>GRN</i>	<i>NPC2</i>	0.006593361	Co-expression	Wang-Maris-2006
<i>MAFK</i>	<i>GM2A</i>	0.012908783	Co-expression	Wang-Maris-2006
<i>LY96</i>	<i>LY86</i>	0.009755008	Co-expression	Mallon-McKay-2013
<i>HSD3B1</i>	<i>GM2A</i>	0.012744123	Co-expression	Mallon-McKay-2013
<i>HSD3B1</i>	<i>CRH</i>	0.011914967	Co-expression	Mallon-McKay-2013
<i>HSD3B1</i>	<i>LHB</i>	0.011909817	Co-expression	Mallon-McKay-2013
<i>ST8SIA4</i>	<i>GM2A</i>	0.02893034	Co-expression	Bild-Nevins-2006 B
<i>MAFK</i>	<i>GM2A</i>	0.021602744	Co-expression	Bild-Nevins-2006 B
<i>GRN</i>	<i>GM2A</i>	0.020527184	Co-expression	Burington-Shaughnessy-2008
<i>AMFR</i>	<i>GM2A</i>	0.020826323	Co-expression	Burington-Shaughnessy-2008
<i>ST8SIA4</i>	<i>CD40</i>	0.01166518	Co-expression	Burington-Shaughnessy-2008
<i>GAA</i>	<i>GRN</i>	0.021335509	Co-expression	Burington-Shaughnessy-2008
<i>CD40</i>	<i>GM2A</i>	0.026018724	Co-expression	Dobbin-Giordano-2005
<i>ST8SIA4</i>	<i>GM2A</i>	0.02396974	Co-expression	Dobbin-Giordano-2005

<i>ST8SIA4</i>	<i>LY86</i>	0.01011246	Co-expression	Dobbin-Giordano-2005
<i>LY86</i>	<i>GM2A</i>	0.014090131	Co-expression	Bahr-Bowler-2013
<i>GRN</i>	<i>NPC2</i>	0.003417451	Co-expression	Bahr-Bowler-2013
<i>GAA</i>	<i>NPC2</i>	0.008345172	Co-expression	Bahr-Bowler-2013
<i>GAA</i>	<i>GRN</i>	0.007346965	Co-expression	Bahr-Bowler-2013
<i>LY96</i>	<i>LY86</i>	0.006099948	Co-expression	Innocenti-Brown-2011
<i>GRN</i>	<i>LY86</i>	0.006802562	Co-expression	Innocenti-Brown-2011
<i>GRN</i>	<i>NPC2</i>	0.01666259	Co-expression	Innocenti-Brown-2011
<i>FABP5</i>	<i>LY96</i>	0.011711845	Co-expression	Innocenti-Brown-2011
<i>LY96</i>	<i>NPC2</i>	0.006187806	Co-expression	Rieger-Chu-2004
<i>GRN</i>	<i>GM2A</i>	0.010561576	Co-expression	Rieger-Chu-2004
<i>GRN</i>	<i>NPC2</i>	0.005401576	Co-expression	Rieger-Chu-2004
<i>GRN</i>	<i>LY96</i>	0.003754711	Co-expression	Rieger-Chu-2004
<i>FABP5</i>	<i>GM2A</i>	0.014316405	Co-expression	Roth-Zlotnik-2006
<i>GAA</i>	<i>GM2A</i>	0.010570861	Co-expression	Perou-Botstein-2000
<i>ALPP</i>	<i>GPLD1</i>	0.003473165	Co-expression	Smirnov-Cheung-2009
<i>CRH</i>	<i>ALPP</i>	0.004937839	Co-expression	Wang-Cheung-2015
<i>GRN</i>	<i>HEXA</i>	0.01568475	Co-expression	Chen-Brown-2002
<i>GRN</i>	<i>NPC2</i>	0.016359169	Co-expression	Chen-Brown-2002
<i>HSD3B1</i>	<i>AMFR</i>	0.018177362	Co-expression	Chen-Brown-2002
<i>GAA</i>	<i>HEXA</i>	0.01394323	Co-expression	Chen-Brown-2002
<i>GAA</i>	<i>GRN</i>	0.012652976	Co-expression	Chen-Brown-2002
<i>PRL</i>	<i>HSD3B1</i>	0.019465497	Co-expression	Wu-Garvey-2007
<i>STS</i>	<i>GM2A</i>	0.030206537	Co-localization	Schadt-Shoemaker-2004
<i>CD40</i>	<i>GM2A</i>	0.028133877	Co-localization	Schadt-Shoemaker-2004

<i>GRN</i>	<i>GM2A</i>	0.014236832	Co-localization	Schadt-Shoemaker-2004
<i>GRN</i>	<i>HEXA</i>	0.009402739	Co-localization	Schadt-Shoemaker-2004
<i>GRN</i>	<i>STS</i>	0.013237921	Co-localization	Schadt-Shoemaker-2004
<i>GRN</i>	<i>CD40</i>	0.008915158	Co-localization	Schadt-Shoemaker-2004
<i>ALPP</i>	<i>GM2A</i>	0.025433496	Co-localization	Schadt-Shoemaker-2004
<i>ALPP</i>	<i>STS</i>	0.025644204	Co-localization	Schadt-Shoemaker-2004
<i>FABP5</i>	<i>GM2A</i>	0.02687657	Co-localization	Schadt-Shoemaker-2004
<i>FABP5</i>	<i>CD40</i>	0.027478961	Co-localization	Schadt-Shoemaker-2004
<i>AMFR</i>	<i>GM2A</i>	0.021205518	Co-localization	Schadt-Shoemaker-2004
<i>AMFR</i>	<i>ALPP</i>	0.017865056	Co-localization	Schadt-Shoemaker-2004
<i>AMFR</i>	<i>FABP5</i>	0.013939995	Co-localization	Schadt-Shoemaker-2004
<i>CRH</i>	<i>GM2A</i>	0.0229119	Co-localization	Schadt-Shoemaker-2004
<i>CRH</i>	<i>STS</i>	0.024494056	Co-localization	Schadt-Shoemaker-2004
<i>CRH</i>	<i>ALPP</i>	0.020280294	Co-localization	Schadt-Shoemaker-2004
<i>CRH</i>	<i>FABP5</i>	0.022545591	Co-localization	Schadt-Shoemaker-2004
<i>LHB</i>	<i>GM2A</i>	0.021348838	Co-localization	Schadt-Shoemaker-2004
<i>LHB</i>	<i>ALPP</i>	0.025315747	Co-localization	Schadt-Shoemaker-2004
<i>LHB</i>	<i>FABP5</i>	0.022165282	Co-localization	Schadt-Shoemaker-2004
<i>LHB</i>	<i>AMFR</i>	0.013310601	Co-localization	Schadt-Shoemaker-2004
<i>LHB</i>	<i>CRH</i>	0.023500843	Co-localization	Schadt-Shoemaker-2004
<i>HSD3B1</i>	<i>GM2A</i>	0.01956358	Co-localization	Schadt-Shoemaker-2004
<i>HSD3B1</i>	<i>ALPP</i>	0.018070487	Co-localization	Schadt-Shoemaker-2004
<i>HSD3B1</i>	<i>AMFR</i>	0.017620761	Co-localization	Schadt-Shoemaker-2004
<i>HSD3B1</i>	<i>LHB</i>	0.021880075	Co-localization	Schadt-Shoemaker-2004
<i>PRL</i>	<i>GM2A</i>	0.026748382	Co-localization	Schadt-Shoemaker-2004

<i>PRL</i>	<i>STS</i>	0.024578847	Co-localization	Schadt-Shoemaker-2004
<i>PRL</i>	<i>ALPP</i>	0.023220547	Co-localization	Schadt-Shoemaker-2004
<i>PRL</i>	<i>FABP5</i>	0.027938604	Co-localization	Schadt-Shoemaker-2004
<i>PRL</i>	<i>AMFR</i>	0.024674127	Co-localization	Schadt-Shoemaker-2004
<i>PRL</i>	<i>CRH</i>	0.031325996	Co-localization	Schadt-Shoemaker-2004
<i>PRL</i>	<i>LHB</i>	0.024771485	Co-localization	Schadt-Shoemaker-2004
<i>GAA</i>	<i>GM2A</i>	0.021490874	Co-localization	Schadt-Shoemaker-2004
<i>GAA</i>	<i>HEXA</i>	0.015766772	Co-localization	Schadt-Shoemaker-2004
<i>GAA</i>	<i>GRN</i>	0.011875112	Co-localization	Schadt-Shoemaker-2004
<i>GAA</i>	<i>ALPP</i>	0.016947022	Co-localization	Schadt-Shoemaker-2004
<i>GAA</i>	<i>AMFR</i>	0.021210423	Co-localization	Schadt-Shoemaker-2004
<i>GAA</i>	<i>PRL</i>	0.022300243	Co-localization	Schadt-Shoemaker-2004
<i>LY96</i>	<i>LY86</i>	0.006775627	Co-localization	Johnson-Shoemaker-2003
<i>STS</i>	<i>GM2A</i>	0.015290882	Co-localization	Johnson-Shoemaker-2003
<i>GRN</i>	<i>GM2A</i>	0.017155321	Co-localization	Johnson-Shoemaker-2003
<i>ALPP</i>	<i>GM2A</i>	0.01002972	Co-localization	Johnson-Shoemaker-2003
<i>ALPP</i>	<i>TFAP2A</i>	0.009357058	Co-localization	Johnson-Shoemaker-2003
<i>ALPP</i>	<i>STS</i>	0.008613536	Co-localization	Johnson-Shoemaker-2003
<i>CRH</i>	<i>GM2A</i>	0.008725192	Co-localization	Johnson-Shoemaker-2003
<i>CRH</i>	<i>TFAP2A</i>	0.008326863	Co-localization	Johnson-Shoemaker-2003
<i>CRH</i>	<i>STS</i>	0.007740852	Co-localization	Johnson-Shoemaker-2003
<i>CRH</i>	<i>ALPP</i>	0.005179046	Co-localization	Johnson-Shoemaker-2003
<i>LHB</i>	<i>GM2A</i>	0.008484612	Co-localization	Johnson-Shoemaker-2003
<i>LHB</i>	<i>TFAP2A</i>	0.008305473	Co-localization	Johnson-Shoemaker-2003
<i>LHB</i>	<i>STS</i>	0.00752627	Co-localization	Johnson-Shoemaker-2003

<i>LHB</i>	<i>ALPP</i>	0.005130447	Co-localization	Johnson-Shoemaker-2003
<i>LHB</i>	<i>CRH</i>	0.004576681	Co-localization	Johnson-Shoemaker-2003
<i>MAFK</i>	<i>GM2A</i>	0.010018589	Co-localization	Johnson-Shoemaker-2003
<i>MAFK</i>	<i>TFAP2A</i>	0.009307076	Co-localization	Johnson-Shoemaker-2003
<i>MAFK</i>	<i>STS</i>	0.007400996	Co-localization	Johnson-Shoemaker-2003
<i>MAFK</i>	<i>ALPP</i>	0.005228889	Co-localization	Johnson-Shoemaker-2003
<i>MAFK</i>	<i>CRH</i>	0.004663254	Co-localization	Johnson-Shoemaker-2003
<i>MAFK</i>	<i>LHB</i>	0.004635511	Co-localization	Johnson-Shoemaker-2003
<i>GAA</i>	<i>HEXA</i>	0.014947882	Co-localization	Johnson-Shoemaker-2003
<i>GAA</i>	<i>GRN</i>	0.016629409	Co-localization	Johnson-Shoemaker-2003
<i>LY96</i>	<i>LY86</i>	0.1184115	Pathway	Wu-Stein-2010
<i>TFAP2A</i>	<i>GM2A</i>	0.082734935	Pathway	Wu-Stein-2010
<i>JUN</i>	<i>GM2A</i>	0.061802242	Pathway	Wu-Stein-2010
<i>ALPP</i>	<i>TFAP2A</i>	0.12819351	Pathway	Wu-Stein-2010
<i>LY96</i>	<i>LY86</i>	0.1488301	Pathway	REACTOME
<i>LY96</i>	<i>LY86</i>	0.70710677	Physical Interactions	IREF-DIP
<i>JUN</i>	<i>TFAP2A</i>	0.069114916	Physical Interactions	Li-Chen-2015
<i>HEXA</i>	<i>GM2A</i>	1	Physical Interactions	BIOGRID-SMALL-SCALE-STUDIES
<i>MAFK</i>	<i>JUN</i>	0.03714322	Physical Interactions	IREF-BIND
<i>HEXA</i>	<i>GM2A</i>	0.77715117	Physical Interactions	IREF-HPRD
<i>GPLD1</i>	<i>GM2A</i>	0.23492727	Physical Interactions	IREF-HPRD
<i>ALPP</i>	<i>GPLD1</i>	0.21245266	Physical Interactions	IREF-HPRD
<i>HEXA</i>	<i>GM2A</i>	0.41421357	Predicted	Wu-Stein-2010
<i>GPLD1</i>	<i>GM2A</i>	0.76536685	Predicted	Wu-Stein-2010

<i>LY86</i>	<i>GM2A</i>	0.1993388	Shared protein domains	INTERPRO
<i>NPC2</i>	<i>GM2A</i>	0.1993388	Shared protein domains	INTERPRO
<i>NPC2</i>	<i>LY86</i>	0.054153882	Shared protein domains	INTERPRO
<i>LY96</i>	<i>GM2A</i>	0.19933881	Shared protein domains	INTERPRO
<i>LY96</i>	<i>LY86</i>	0.054153886	Shared protein domains	INTERPRO
<i>LY96</i>	<i>NPC2</i>	0.054153886	Shared protein domains	INTERPRO
<i>ALPP</i>	<i>STS</i>	0.018784788	Shared protein domains	INTERPRO
<i>MAFK</i>	<i>JUN</i>	0.025053639	Shared protein domains	INTERPRO
<i>GAA</i>	<i>HEXA</i>	0.009137378	Shared protein domains	INTERPRO
<i>LY86</i>	<i>GM2A</i>	0.33333334	Shared protein domains	PFAM
<i>NPC2</i>	<i>GM2A</i>	0.33333334	Shared protein domains	PFAM
<i>NPC2</i>	<i>LY86</i>	0.33333334	Shared protein domains	PFAM
<i>LY96</i>	<i>GM2A</i>	0.33333334	Shared protein domains	PFAM
<i>LY96</i>	<i>LY86</i>	0.33333334	Shared protein domains	PFAM
<i>LY96</i>	<i>NPC2</i>	0.33333334	Shared protein domains	PFAM

4. DISCUSSION:

According to our analysis we found four SNPs in *GM2A* gene possibly regarded as highly damaging SNPs which they are: C99Y, C112F, C138S and C138R. All of them are novel SNPs except C138R which is reported in previous studies. Sheth et al (2016) and Kochumon et al (2017) studies are agree with us that C138R found in *GM2A* gene and associated with AB variant of GM2 gangliosidosis (22, 45).

Regarding C99Y (rs751417546) SNP, it has differences between wild-type and mutant residue in size and hydrophobicity; according to size, mutant residue is bigger than the wild-type residue that is located on the surface of the protein so mutation of this residue can disturb interactions with other molecules or other parts of the protein. On other hand, the wild-type residue is more hydrophobic than the mutant residue and the mutation might cause loss of hydrophobic interactions with other molecules on the surface of the protein. In case of C112F (rs773743799)

SNP, the wild type residue is bigger than mutant residue also it buried in the core of a domain so the differences between the wild and mutant residue might disturb the core structure of this domain. Concerning C138S (rs1174735558) SNP, the wild-type residue is more hydrophobic than the mutant residue. The mutation might cause loss of hydrophobic interactions with other molecules on the surface of the protein. The mutation is located in a region with known splice variants. A mutation to "Arginine" was found at this position as C138R (rs137852797) which has dissimilarities between wild-type and mutant amino acids in size, charge and hydrophobicity. As the wild-type residue charge was NEUTRAL, the mutant residue charge is POSITIVE. The mutation introduces a charge at this position; this can cause repulsion between the mutant residue and neighboring residues. And According to size, the mutant residue is bigger than the wild-type residue. The residue is located on the surface of the protein; mutation of this residue can disturb interactions with other molecules or other parts of the protein. On other hand, the wild-type residue is more hydrophobic than the mutant residue. The mutation might cause loss of hydrophobic interactions with other molecules on the surface of the protein.

The wild-type residues of the four SNPs are annotated to be involved in a cysteine bridge, which is important for stability of the protein, only cysteines can make this type of bonds. The mutation causes loss of this interaction and will have a severe effect on the 3D-structure of the protein. Together with loss of the cysteine bond, the differences between the old and new residue can cause destabilization of the structure. Also the wild-type residues are extremely conserved, based on conservation scores these mutations are probably damaging to the protein.

46 SNPs out of 676 SNPs were predicted to affect miRNAs binding sites on 3'UTR leading to abnormal expression of the resulting protein. 112 alleles disrupt a conserved miRNAs binding sites while 57 derived alleles creating a new binding site of miRNAs.

GM2A gene function and activities illustrated by GENEMANI as following: glycolipid metabolic process, glycosphingolipid metabolic process, membrane lipid metabolic process, sphingolipid metabolic process, vacuolar part.

As the great role of using bioinformatics tools, laboratory analysis accompanied with in vivo analysis remains highly recommended confirming our findings. These findings could be used in a helpful way to improve the diagnosis and screening of AB variant of GM2 gangliosidosis.

5. CONCLUSION:

Through using different bioinformatics tools, four SNPs were found to be highly damaging SNPs that affect function, structure and stability of *GM2A* protein, which they are: C99Y, C112F, C138S and C138R, three of them are novel SNPs (C99Y, C112F and C138S). Also 46 SNPs were predicted to affect miRNAs binding sites on 3'UTR leading to abnormal expression of the resulting protein. 112 alleles disrupt a conserved miRNAs binding sites while 57 derived alleles creating a new binding site of miRNAs. These SNPs should be considered as important candidates in causing AB variant of GM2 gangliosidosis and may help in diagnosis and genetic screening of the disease.

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7. DATA AVAILABILITY:

All relevant data used to support the findings of this study are included within the manuscript and supplementary information files.

8. CONFLICT OF INTEREST:

The authors declare that there is no conflict of interest regarding the publication of this paper.

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