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Network and Polymorphic Analysis of Obesity Candidate Gene-Plin1: A Bioinformatics Approach

SusmitaBag, Sudha Ramaiah and Anand Anbarasu*

Medical and Biological Computing Laboratory, School of Biosciences and Technology, VIT University, Vellore 632014, Tamil Nadu, India

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ABSTRACT Gene network analys is provides an intricate interrelation between linked network elements. The aim of the present research study is tofocus on compact network associated with key obesity candidate gene, perilipin-1 and its polymorphic study. The methodology constitutes the *in silico* gene network, enrichment analysis and polymorphic study of plin1 gene. The computational gene network analysis suggested that plin1 is strongly associated with abhd5 and prkaca.The enrichment analysis elucidated their requisite involvement in obesity *via*imbalances in lipid metabolic processes. On demonstrating the damaging effect of diseased nsSNP of plin1 shows that the amino acid change for rsID-rs8179071 is from serine to leucine at position 348 is found to be the diseased state and hence associated with obesity. The plin1 gene shows functional interaction with two genes (abhd5 and prkaca) which are associated with obesity. In addition, polymorphic prediction indicated that rsID-rs8179071 is linked with obesity.

INTRODUCTION

Gene network establishes a dynamic framework, providing a sharp comprehension on biological complexity that has a lane from gene level to interaction networks. These associated interacting components have gained increasing interest in network biology (Hood et al. 2004; Hwang et al. 2009; Li et al. 2012).

Gene networks have been set forth to find novel candidate genes, based on the assumption that neighbors of a disease-causing gene in a network are more likely to cause either the same or a similar disease (Segal et al. 2005; Bernardo et al. 2005; Hu et al. 2011).To interpret the roles they play in complex diseases, genes need to be investigated in the networks they are involved in. The interaction network represents precise information than lists of genes or pathways, as it describes which genes are closely connected within a given pathway. Also a gene network envisages a gene products interactive network, that helps in identifying potential roles for proteins associated with core sophisticated processes and functions. Hence, network analysis has the potential to detect more elusive signals, such as local disturbances within known pathways, as well as within pathways that have not yet been described. Gene networks can be constructed by ensembling of previously reported interactions in the literature and various databases like the database of interacting proteins (DIP) (Xenarios et al. 2000), the biomolecular interaction network database (BIND) (Alfaranoet al. 2005), the BioGRID (Aryamontri et al. 2013), human protein reference database (HPRD) (Keshava et al. 2009) and IntAct (Kerrien et al. 2012). Network study provides unified information originating from multiple studies and to identify key components in a system (Hayasaka et al. 2011).

In the recent network-based analysis, the researchers demonstrated a complex interplay of plin1 in association with its shared partner and representing functional relationships among genes in plin1 network, for the purpose of understanding gene function in plin1 network and obesity. Obesity is found to be a global issue as mentioned by World Health Organization (WHO) (WHO Technical Report Series, Series 894, 2000; Zimmet and Alberti 2006; Ng et al. 2014). Obesity is also the result of a composite web of hundreds of genes and scores of environmental factors potentially drawn in its expression. It is a multifactor disorder which not only manifest genetic basis but also requires environmental influences (Qi and Cho 2008).

Amongst different environmental/behavioral factors implicated in obesity, the most signif-

*Address for correspondence:
Dr. Anand Anbarasu
Professor
VIT University
Tamil Nadu, India
Telephone: +91-416-2202547
Fax:+91-416-2243092
E-mail: aanand@vit.ac.in

icant are probably diet and physical activity. It has been observed that, both excess caloric intake and insufficient physical activity put in to the accumulation of energy in the body in the form of adipose tissue. These rate of accumulation and/or release of that excess of energy stored in the adipocyte varies considerably among individuals and several genes have been suggested to be involved in these processes. Among them, we can highlight the perilipin family of genes, encoding for the most abundant proteins surrounding the lipid droplet in the adipocyte. The gene, plin1 is an important obesity candidate and lipid droplet associated gene in humans and has its potential usefulness in identifying those predisposed to obesity and to estimate the relative risk of obesity (Ordovás and Smith 2010; Smith and Ordovás 2012; Patel et al. 2014). Here, the researchers attempted a gene network and a biochemical analysis with plin1 to find out the involvement of plin1. The present results will be helpful for researchers in the field of obesity and related disorders.

Objectives

- Gene network for plin1 is constructed.
- The plin1 gene strongly interacts with two functional genesabhydrolase domain containing 5 (abhd5) and protein kinase, cAMP-dependent, catalytic, alpha (prkaca), which is associated with obesity.
- The plin1 plays a central role in obesity candidate and lipid droplet associated gene in humans.
- The computational predictions of nsSNP have a deleterious effect on plin1 and result in obesity associated metabolic disorder.

METHODOLOGY

Tools for Construction of plin1 Functional Modules

For functional identification of modules associated with plin1 several tools were utilized for coding the functional genes associated with plin1 that include: the search tool for retrieval of interacting genes/proteins (STRING 9.0) (Snel et al. 2000; Meringet al. 2003; Meringet al. 2005; Meringet al. 2007; Jensen et al. 2009; Szklarczyk et al. 2011), GeneMANIA (Warde et al. 2010), Cognoscente (Datta et al. 2009), a molecular inter-relations in the integrative network is obtained using cytoscape plugin: BisoGenet (Martin et al. 2010) followed by Pathway Common Web Service Client (Cerami et al. 2011) for biochemical analysis.

Network Visualization

The network was visualized and constructed using cytoscape version 2.7.0 and cytoscape version 3.0.2 (Shannon et al. 2003). Cytoscape supported several algorithms for the layout of networks which included spring-embedded layout, hierarchical layout, circular layout and attribute-based layout. In fact, Cytoscape was used to represent graphical network layout model for our work (Shannon et al. 2003).

Gene Ontology Analysis and SNP Analysis

Performing the functional ontological analysis of the candidate genes, we used WebGestalt (Jia et al. 2011) for Gene Ontology (GO) term analysis. Briefly, WebGestalt implements the hypergeometric test for the enrichment of GO terms in the candidate genes, followed by the correction of multiple testing using the Benjaminiand Hochberg (BH) method (Jia et al. 2011).

Later, the gene network and enrichment analysis was linked up with non-synonymous SNP analysis of plin1. For finding out nsSNPs of plin1, functional single nucleotide polymorphism (F-SNP) database (Lee and Shatkay 2008) was used. The F-SNP database identified nsSNPs that had deleterious effects on protein structure or function or hinder post-translational modifications (Lee and Shatkay 2008). To predict the functional effects of nsSNPs in protein coding region several tools were mined that include Sorting Intolerant from Tolerant (SIFT) (Ng and Henikoff 2003; Shen et al. 2006), Polymorphism Phenotyping (PolyPhen) (Johnson et al. 2005; Zhu et al. 2008). For locating SNPs in exonic-splice regions, following F-SNP tools were used: Exonic splicing enhancer (ESEfinder) (Cartegni et al. 2003), relative enhancer and silencer classification by unanimous enrichment (RESCUE-ESE) (Fairbrother et al. 2004), exonic splicing regulator (ESRSearch) (Fairbrother et al. 2002) and putative exonic splicing enhancer (PESE) (Zhang et al. 2005). In addition, OGPET (Gerken et al. 2004) was used to examine post-translational modification sites. OGPET predicted the O-gly-

cosylation sites (Lanver et al. 2010). Last but not the least, for exploring the phenotypic effect of nsSNPs, SNPs & GO (Calabrese et al. 2009) was used.

RESULT AND DISCUSSION

A systematic network study for finding emergent properties of human gene-gene/gene-disease networks by means of network interconnecting patterns and functional annotation analysis indicate a highly shared association of plin1 with genes involved in obesity related disorders.

Network and Functional Significance of Selected Modules in plin1 Network

In the present research study, two interactive network modules for plin1 have been obtained from STRING and GeneMANIA. Network of twenty genes, tabulated in Table 1, are extracted from both STRING and GeneMANIA. Besides, among these twenty genes, six genes are found to be common, which have been retrieved from STRING and GeneMANIA (abhd5, fabp4, lipe, prkacg, cav1 and rxra).

In STRING, functional interaction is analyzed by using confidence score ranging from 0.5 to 1.0. Interactions with score < 0.3 are considered as low confidence, scores ranging from 0.3 to 0.7 are classified as medium confidence and score > 0.7 yield high confidence (Li et al. 2009; Szklarczyk et al. 2011). The gene web of plin1, with respect to STRING, constitutes abhd5, fabp4, lipe, prkacg, cav1 and rxra. They are found to be linked to the main node: plin1, with a high confidence score of 0.89 or above, as mentioned in Table 2.

All the six genes associated with plin1 present text mining evidence. Here, the first two interaction partners associated with plin1 are abhd5 and prkaca. It has been observed that, abhd5 has acyltransferase activity for the synthesis of phosphatidic acid, the major intermediate in membrane and storage lipid biosynthesis and acts as a coactivator of adipocyte triglyceride lipase (ATGL), both activities being independent (Ghosh et al. 2008). Also abhd5, a lipid-

Table 1: Linked genes associated in plin1 network obtained from STRING andGeneMANIA

S.No.	*Genes associated with plin1 obtained from GeneMANIA*	*Genes associated with plin1 obtained from STRING 9.05*
1.	abhd5 (abhydrolase domain containing 5)	abhd5 (abhydrolase domain containing 5)
2.	cav1 (caveolin 1, caveolae protein)	cav1 (caveolin 1, caveolae protein)
3.	fabp4 (fatty acid binding protein 4, adipocyte)	fabp4 (fatty acid binding protein 4, adipocyte)
4.	rxra (retinoid x receptor, alpha)	rxra (retinoid x receptor, alpha)
5.	lipe (lipase, hormone-sensitive)	lipe (lipase, hormone-sensitive)
6.	prkacg (protein kinase, camp-dependent, catalytic, gamma)	prkacg (protein kinase, camp-dependent, catalytic, gamma)
7.	pparg (peroxisome proliferator-activated receptor gamma)	pparg (peroxisome proliferator-activated receptor gamma)
8.	adipoq (adiponectin, c1q and collagen domain containing)	cdk8 (cyclin-dependent kinase 8)
9.	aqp7 (aquaporin 7)	ccnc (cyclin c)
10.	plin4 (perilipin 4)	cdk19 (cyclin-dependent kinase 19)
11.	plin5 (perilipin 5)	med12 (mediator complex subunit 12)
12.	plin2 (perilipin 2)	med13 (mediator complex subunit 13)
13.	plin3 (perilipin 3)	med14 (mediator complex subunit 14)
14.	ppp1ca (protein phosphatase 1, catalytic subunit, alpha isozyme)	med18 (mediator complex subunit 18)
15.	ppp1cb (protein phosphatase 1, catalytic subunit, beta isozyme)	med29 (mediator complex subunit 29)
16.	pck1 (phosphor-enol-pyruvate-carboxy-kinase 1 (soluble))	ncoa2 (nuclear receptor coactivator 2)
17.	g0s2 (g0/g1switch 2)	ncoa3 (nuclear receptor coactivator 3)
18.	gpd1 (glycerol-3-phosphate dehydrogenase 1 (soluble))	ncoa3 (nuclear receptor coactivator 3)
19.	rbp4 (retinol binding protein 4, plasma)	pnpla2 (patatin-like phospholipase domain containing 2)
20.	saa1 (serum amyloid a1)	ppargc1a (peroxisome proliferator-activated receptor gamma)

Table 2: STRING interaction of plin1 with functional partners

S.No.	*Functional node*	*Interaction type*	*Types of evidence for the association*	*Combined confidence score*
1.	abhd5	Binding Biochemical Reaction	Experimental/Biochemical Data (Yamaguchi et al. 2004), Association in Curated Databases (Reactome and pathway interaction database), Co-Mentioned in PubMed abstracts (Ghosh et al. 2008; Yamaguchi et al. 2004)	0.995
2.	fabp4	nil	Co-Expression, Association in Curated Databases (Reactome, KEGG), Co-Mentioned in PubMed abstracts (Krawczyk et al. 2012)	0.964
3.	lipe	Binding, post-translational modification	Co-Expression, Association in Curated Databases(Reactome), Co-Mentioned in PubMed abstracts (Mak 2012)	0.995
4.	prkacg	Catalysis	Association in Curated Databases (Reactome)	0.899
5.	cav1	NIL	Association in Curated Databases (Reactome, Pathway interaction database), Co-Mentioned in PubMed abstracts (Bourez et al. 2012)	0.928
6.	rxra	NIL	Association in Curated Databases (Reactome, KEGG), Co-Mentioned in PubMed Abstracts (D'Ambrosio et al. 2011)	0.907

droplet-associated protein is characterized by excess lipid storage, activates adipose triglyceride lipase and thus may regulate fat mobilization (Lass et al. 2006; Cantley et al. 2013). Mutations of this gene cause a rare lipid storage disease that is characterized by accumulation of triglycerides in the cytoplasm of leukocytes, muscle, liver, fibroblasts and other tissues (Ghosh et al. 2008; Yamaguchi et al. 2004). The gene, prkacg is involved in the regulation of lipid and glucose metabolism (Zhang et al. 2004; Berrabah et al. 2011). Alternatively spliced transcript variants encoding distinct isoforms have been observed. The fabp4 encodes the fatty acid binding protein found in adipocytes, and its roles include fatty acid uptake, transport, and metabolism (Krawczyk et al. 2012). Also it is mined that, lipe is expressed in adipose tissue, helps in hydrolyses of stored triglycerides to free fatty acids (Mak 2012). A scaffolding protein, cav1 is the main component of the caveolae plasma membranes found in most cell types, which links integrin subunits to the tyrosine kinase 'fyn', an initiating step in promoting cell cycle progression. It is a tumor suppressor gene candidate and forms a stable hetero-oligomeric complex with cav2. Mutations in this gene have been associated with Berardinelli-Seip congenital lipodystrophy (Bourez et al. 2012). It interacts directly with G-protein alpha sub-units and can functionally regulate their activity. It is also involved in the co-stimulatory signal essential for TCR mediated T-cell activation. The retinoic acid receptors, rxra's, are nuclear receptors that bind as heterodimers to their target response elements in response to their ligands and mediate the biological effects of retinoids by their involvement in retinoic acid-mediated gene activation (D'Ambrosio et al. 2011). Greater methylation on the rxra gene was associated with greater fat levels in later childhood (D'Ambrosio et al. 2011).

The present work is also clasped with GeneMANIA framework, where all the six genes (abhd5, fabp4, lipe, prkacg, cav1, and rxra) are found to be associated with plin1 network and thus resulted with the same interacted partners as obtained in STRING results. GeneMANIA works on the basis of weighted network framework. Each and every interaction associated with the plin1 gene and its interacted partners, is assigned by a weight on the basis of association, thus explaining functional similarities network that summarizes all of the evidence of co-functionality. Table 3 explains weighted percentage network associated with plin1.

Identification of Key Functional Node Associated with plin1 Network

In the research, after the selection of interactive modules in plin1 network, certain online resources: COGNOSCENTE (Datta et al. 2009) and BISOGENET (Martin et al. 2010) are employed for the analysis of genes which are strongly as-

Table 3: Weighted network percentage associated with plin1 and its partners

S.No.	*Associated partner with plin1*	*Types of interaction*	*Percent weight*
1.	abhd5	physical interactions(protein-protein interaction)	67.1
1.		Pathway (Wu et al. 2010)	66.2
2.	fabp4	co-expression 4.4	
3.	lipe	co-expression 2.0	
4.	prkacg	Pathway (Wu et al. 2010)	3.7
5.	cav1	co-expressionpathway (Wu et al. 2010)	1.06.3
6.	rxra	co-localizationpathway (Wu et al. 2010)	3.02.0

sociated in the plin1 network. The main aspect of the following study is to locate the highly functionally enriched moieties that are associated with plin1. These modules may be valuable for identifying candidate genes related to multipart traits and should be investigated further.

Further, among the six commonly interacted genes in plin1 network, it has been observed that two genes (abhd5 and prkaca) are found to be highly functionally associated with plin1. Starting with the computational analysis for illustrating the highly associated functional genes, the two commonly allied functional genes (abhd5 and prkaca) associated in plin1 interaction network, are obtained from cognoscente database. Interactions represented in cognoscente include protein-protein, protein-DNA and genetic interactions. Here, the researchersincorporated the query gene, plin1, which provides a list of interactions, as mentioned in Table 4, explaining that abhd5 and prkaca are the two gene products that show a protein-protein interaction with plin1.

To evaluate further, the interaction of queried gene, plin1, with commonly associated functional partners (abhd5 and prkaca), we employed BisoGenet for analyzing biological network. The result drawn out for plin1 network from BisoGenet involves a graph based search operation and has been tabulated in Table 5. Further, Table 5demonstrates that there is a protein-protein interaction of plin1, respectively with abhd5 and prkaca. Thus the researchers suggest that plin1 follows a highly co-functional network with abhd5 and prkaca (Patel et al. 2014).

The network study also provides a biochemical pathway analysis for plin1 by "pathway common web service client". To reduce the ambiguity among the gene identifiers, a systematic pathway study is done for plin1 system study. To find out an exact interactive mechanism among plin1, abhd5 and prkaca, a full Biopax representation of plin1 network is obtained from cytoscape 3.0.2. The system involves a cytosolic mechanism, where an organized manner of lipids and lipoprotein metabolism takes place. The

Table 4: Interaction for plin1 obtained from cognoscent

Cognoscente id	*Gene symbol 1*	*Gene symbol 2*	*Interaction types*	*Original DB source*
408695	plin1	prkaca	protein-protein	HRPD
408696	plin1	abhd5	protein-protein	HRPD

Table 5: Biso Genet result for PLIN1

S.No.	*Bio-relation description (protein –protein interaction)*	*Experimental method*	*Database*	*Pubmed ID*
1.	Interactor A: abhd5	HPRD: 11986, 01364	two in vitro	hybrid, 15136565
	Interactor B: plin1	HPRD: 11986, 01364	two vitro	
2.	Interactor A: prkaca Interactor B: plin1	HPRD: 03382, 01364	In vitro	11751901

interactive plin1 network includes biochemical reactions for adipose tissue lipolysis involving protein, its complex and small molecules. To the surface of cytosolic lipid particle, plin1 is localized as a complex (perilipin: CGI-58 complex) with abhd5 (CGI-58 abbreviated as Comparative Gene Identification-58). It has been proved that from the body's natural lipases, such as hormone-sensitive lipase perilipin acts as a protective coating, which breaks triglycerides into glycerol and free fatty acids for the use in metabolism, a process known as lipolysis (Gautam et al.2013; Patel et al. 2014). The two associated lipases include: i) Adipose triglyceride lipase (ATGL) is localized partially to the lipid droplet (LD) and ii) Hormone-sensitive lipase (HSL) present mostly in the cytoplasm. During the stimulated lipolysis process, there is dissociation of perilipin: CGI-58 complex. Later, perilipin, which is a major protein at the surfaces of cytosolic lipid particles in adipocytes and steroidogeniccells (Blanchette et al. 1995; Thiam et al. 2013) and HSL is phosphorylated by protein-kinase-A, catalytic subunit of prkaca. This above mechanism causes releases of CGI-58, followed by fragmentation of LD. Phosphorylation of perilipin and HSL, involves the addition of two molecules of *adenosine triphosphate (ATP)*, and thus facilitates *adenosine diphosphate (ADP)* release. On phosphorylation of perilipin releases CGI-58, which binds ATGL to initiate lipolysis and followed by translocation of HSL to the LD and degrades diglyceride. After the translocation process, dephosphorylation of HSL and perilipin takes place, thus releasing orthophosphate, resulting in relocalization of HSL in cytoplasm and perilipin in LD.

Crosstalk of Linked

Gene Ontology Enrichment Analysis

To emphasize on functional associations between gene products, we constructed a geneco annotation network based on the significant terms in the biological processes, molecular function and cellular component of GO using three nodes (plin1, abhd5 and prkaca) of the core network. These specific functional features are performed using web based gene set analysis toolkit (WebGestalt version 2.0).

A hierarchical ontological analysis for plin1, abhd5 and prkaca has been studied. We found that these three genes are associated with most significant biological metabolic processes. All the GO terms categories (BP: biological process; MF: molecular function; and CC: cellular component) are mentioned in Tables 6, 7 and 8. The highly significant terms obtains are biological processes and these terms are frequently annotated to the entire three genes.

Biological processes obtained (Table 6) explains that triglyceride catabolic process is significantly and highly enriched process, with a p-

Table 6: Biological processes involved in plin1 network

GO term	*Genes associated*	*rawP*	*adjP*
Triglyceride catabolic process (ID:GO:0019433)	3	3.54e-09	4.86e-07
Acylglycerol catabolic process (ID:GO:0046464)	3	4.05e-09	4.86e-07
Neutral lipid catabolic process (ID:GO:0046461)	3	4.05e-09	4.86e-07
Glycerolipid catabolic process (ID:GO:00465)	3	1.09e-08	9.81e-07
Neutral lipid metabolic process (ID:GO:0006638)	3	4.32e-07	2.22e-05
Triglyceride metabolic process (ID:GO:0006641)	3	3.64e-07	2.22e-05
Acylglycerol metabolic process (ID:GO:0006639)	3	4.20e-07	2.22e-05
Cellular lipid catabolic process (ID:GO:0044242)	3	1.04e-06	4.68e-05
Lipid catabolic process (ID:GO:0016042)	3	5.27e-06	0.0002
Glycerolipid metabolic process (ID:GO:0046486)	3	1.01e-05	0.0004

Table 7: Molecular functions involved in plin1 network

GO term	*Genes associated*	*rawP*	*adjP*
Lysophospholipidacyltransferase activity(ID:GO:0071617)	abhd5	0.0004	0.0096
Lysophosphatidic acid acyltransferase activity (ID:GO:0042171)	abhd5	0.0004	0.0096
1-acylglycerol-3-phosphate O-acyltransferase activity (ID:GO:0003841)	abhd5	0.0018	0.0173
cAMP-dependent protein kinase activity (ID: GO:0004690)	prkaca	0.0018	0.0173
Triglyceride lipase activity(ID:GO:0004806)	abhd5	0.0034	0.0233
Acylglycerol O-acyltransferase activity (ID: GO:0016411)	abhd5	0.0032	0.0233
O-acyltransferase activity(ID:GO:0008374)	abhd5	0.0078	0.0468
Lipase activity(ID:GO:0016298)	abhd5	0.0225	0.1109
Carboxylic ester hydrolase activity(ID:GO:0052689)	abhd5	0.0231	0.1109

Table 8: Cellular functions involved in plin1 network

GO term	*Genes associated*	*rawP*	*adjP*
lipid particle(ID:GO:0005811)	plin1, abhd5	1.85e-05	0.0006
neuromuscular junction (ID:GO:0031594)	prkaca	0.0077	0.0873
microtubule organizing center (ID:GO:0005815)	prkaca	0.0849	0.3226
centrosome(ID:GO:0005813)	prkaca	0.0661	0.0173
perinuclear region of cytoplasm(ID:GO:0048471)	prkaca	0.0817	0.3226
synapse(ID:GO:0045202)	prkaca	0.0854	0.3226
cytoplasmic part(ID:GO:0044444)	prkaca, plin1, abhd5	0.0656	0.3226
cytosol(ID:GO:0005829)	prkaca, abhd5	0.0547	0.3226
microtubule cytoskeleton(ID:GO:0015630)	prkaca	0.1467	0.4936

value of 3.54e-09, common for all the three genes. Later, it has been observed that there are nine other biological processes (acylglycerol catabolic process, neutral lipid catabolic process, glycerolipid catabolic process, neutral lipid metabolic process, triglyceride metabolic process, acylglycerol metabolic process, cellular lipid catabolic process, lipid catabolic process, and glycerolipid metabolic process) which show a common significant association with plin1, abhd5 and prkaca. Hence the analysis sort out a catabolic and anabolic process in lipid molecules that is associated with obesity (Arrese et al. 2014).

From the results presented in Table 7,it is observed that, among the nine molecular functions, seven functions (lysophospholipidacyltransferase activity, lysophosphatidic acid acyltransferase activity, 1-acylglycerol-3-phosphate O-acyltransferase activity, cAMP-dependent protein kinase activity, triglyceride lipase activity, acylglycerol O-acyltransferase activity and O-acyltransferase activity) show significant result. The functions infer that abhd5 is involved in transferase activity and prkaca in kinase activity.

Cellular enrichment analysis reveals that plin1 and abhd5 are localized with lipid particle having highly significant p-value of 0.0006, and are associated with lipolytic mechanism as shown in Table 8. Also it has been observed that, plin1, abhd5 and prkaca are localized in cytosolic part (Arrese et al. 2014).

Phenotypic and Polymorphic Prediction of plin1

An interactive SNP and phenotypic analysis has been carried out, for finding out the disease causing allelic variant associated with the target node, plin1. The plin1 gene investigated for this analysis is predestined for determining the deleterious and damaging effect of non-syn-

onymous single nucleotide polymorphisms (SNP) related with this disease causing gene.

To analyze whether an amino acid substitution at a particular position in a protein molecule will have a phenotypic effect, we used SIFT tool. Amongst fifty-six non-synonymous function class SNP ids (rsIDs'), a prediction of allelic changes associated with twelve rsIDs' (rs114583540, rs112305845, rs111663599, rs74407840, rs58361219, rs56148016, rs17852910, rs8179072, rs8179071, rs8179070, rs6496589 and rs3743373) are obtained from SIFT tool. And among the twelve, five rsIDs' (rs114583540, rs112305845, rs8179072, rs8179071 and rs8179070) are predicted to be damaging due to the following allelic variations R231L, D178N, A386V, S348L and R274W respectively. A descriptive tabulated result has been shown in Table 9.The variations are predicted to be damaging by SIFT program with tolerance index score of <0.05 (Ng and Henikoff 2003; Shen et al. 2006).

Further, the researcher employed PolyPhen tool for SNP analysis and the researcher found that rs8179072 shows a benign effect and the two nsSNP's (rs8179071 and rs8179070) are considered to be possibly damaging effect with position-specific independent count (PSIC) scores of >1.5 (Johnson et al. 2005; Zhu et al. 2008) as elucidated in Table 10.

For further annotation of nsSNP's, F-SNP is used to identify the functional SNPs associated with plin1. The deleterious SNP have a FS score value between 0.5 and 1 (Lee and Shatkay 2009). 3 SNPs (rs8179072, rs8179071 and rs8179070) are found to have FS score 0.908, 0.849 and 0.561 respectively. But except rs8179072, the other two rsID's are found to be deleterious since the two ID's involves the changes in protein-coding region as mentioned in Table 11.Also, changes in splicing regulations region are also found

Table 9: Prediction of nsSNP by SIFT

SNP ID	*Amino acid change*	*Protein*	*Score*	*Prediction*
rs114583540	R231L	NP_001138783	0.04	Damaging
rs112305845	D178N	NP_001138783	0.02	Damaging
rs8179072	A386V	NP_001138783	0.02	Damaging
rs8179071	S348L	NP_001138783	0.01	Damaging
rs8179070	R274W	NP_001138783	0.01	Damaging

Table 10:PolyPhen prediction of nsSNP

SNP ID	*Substitution position*	*First amino acid variant*	*Second amino acid variant*	**PSIC score*	*Prediction*
rs8179072	386	A	V	0.978	benign
rs8179071	348	S	L	1.763	possibly damaging
rs8179070	274	R	W	1.977	possibly damaging

* PSIC: Position Specific Independent Count.

Table 11: Predicted functionalSNPs by F-SNP database

Functional category	*Prediction tool*	*Prediction result*		
		rs8179072 (FS score: 0.908)	*rs8179071 (FS score: 0.849)*	*rs8179070 (FS score: 0.561)*
Protein Coding	PolyPhen	Benign	Possibly damaging	Possibly damaging
	SIFT	Tolerated	Damaging	Damaging
	SNPeffect	Benign	Deleterious	Deleterious
	SNPs3D	Deleterious	Deleterious	Deleterious
	Ensembl	Non-synonymous	Non-synonymous	Non-synonymous
Splicing Regulation	ESEfinder	Changed	Changed	Changed
	ESRsearch	Changed	Changed	Changed
	PESX	Changed	Not changed	Changed
	RESQUE_ESE	Changed	Not changed	Not changed
Post Translation	OGPET	Not exist	Exist	Not exist

Table 12: Diseased nsSNP analysis by SNPs and GO

SNP ID	*Substitution position*	*Residue in wild-type protein*	*Mutated residue*	*Effect*	*Predicted GO terms*
rs8179072	386	A	V	Neutral polymorphism	GO: 0016042 (lipid catabolic process), GO: 0005515 (protein binding),
rs8179071	348	S	L	Disease-related polymorphism	GO: 0008289 (lipid binding),
rs8179070	274	R	W	Neutral polymorphism	GO: 0012511 (monolayer-surrounded lipid storage body)

out for the three SNPs (rs8179072, rs8179071 and rs8179070). Polymorphism associated with diseases interrupts the activity of ESEs, thus dysregulating the core splicing machinery to nearby splice sites (Graveley 2000). Also it has been interestingly observed that the SNPs alter the post-translation modification of protein (Jacoby et al. 2003). Here for plin1, the finding from tool OGPET of F-SNP explains that only one rsID(rs8179071) shows changes in the post-translational site.

Further, on the basis on the basis of the damaging effect of the SNP's, only rs8179071 is taken for polymorphic analysis, as this relevant rsID (rs8179071) shows a possibly damaging effect in protein coding and splicing regulation regions followed by promoting changes in post-translational site as shown in Table 12. And later to verify the associated diseased mutation, SNPs&GO algorithms are used. The SNPs&GO algorithms explore the impact of protein variations using functional information encoded by gene ontology terms: molecular function, biological process and cellular component. From SNPs&GO, The researchers find that the mutation S348L of rsID: rs8179071 is found to be disease associated, and is involved in obesity related traits. The ontology been involved with rs8179071, include GO: 0016042 (lipid catabolic process), GO: 0005515 (protein binding), GO: 0008289 (lipid binding) and GO: 0012511 (monolayer-surrounded lipid storage body) (Arrese et al. 2014). Thus it can be proposed that the mutation at 348th position in plin1 gene having an allele change from serine to leucine, is associated with obesity related phenotype.

CONCLUSION

On the whole, the present research study revealed that, there is a remarkable network association of plin1 with abhd5 and prkaca, allied with lipid metabolic process in obesity. The findings of ontological analysis provide a comprehensible idea on the hierarchal outline of the anabolic and catabolic lipid processes and functions associated with plin1, abhd5 and prkaca. A polymorphic analysis for the seed node-plin1, indicate the diseased allele change from serine to leucine at 348th position and thus rsID-rs8179071 is linked with obesity. The present results gained from this *in silico* study provide a useful insight on plin1 and its role in obesity through gene networks.

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