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In silico Analysis of Functional Single Nucleotide Polymorphisms in Genes Related to Adipose Tissue Impairment

Kaviyarasi Renu[1], Revanius Z. Mbuonu[1], Nishu Sekar[1], Shalaka S Ramgir[1], Yogamaya D Prabhu[1], R. Papitha[2], V. G. Abilash[1]

[1]Department of Biomedical Sciences, School of Biosciences and Technology, VIT University, Vellore, Tamil Nadu, India, [2]Department of Biotechnology, School of Biosciences and Technology, VIT University, Vellore, Tamil Nadu, India

Abstract

Introduction: Adipose tissue is an endocrine organ which is made up of special connective tissue. The major task of this tissue is to accumulate the energy in the form of triacylglycerol (TAG). Adipogenesis is the process of formation of the preadipocyte into the mature adipocyte. Normally adipose tissue is of two types, namely, white adipose tissue (WAT) and brown adipose tissue (BAT). An important role WAT is to store TAG and the significant responsibility BAT is production of heat (thermogenesis). Fat accumulation is identified by the balance between the fat synthesis such as adipogenesis and the fat break down such as lipolysis. When there is excessive amount of intake of food, elevated plasma carbohydrate and triglyceride are involved in lipogenesis in adipose tissue. During fasting condition, there is a process called lipolysis which releases fatty acids and glycerol into the circulation. Our aim is to investigate the single nucleotide polymorphisms (SNP) analysis of genes participated in maintenance of adipose tissue such as hormone sensitive lipase monoglyceride lipase and adiponectin. **Materials and Methods:** *In silico* analysis using sorting intolerant from tolerant (SIFT), polyphen 2 was performed for the genes concerned in maintaining adipose tissue homeostasis. **Results:** SNPs were analyzed for all the genes. Benign and damaging SNPs were identified using SIFT and polyphen 2. **Conclusion:** As SNPs show suppressed stability, damaging and benign character, they can be further utilized for *in vivo* studies to determine the role of these genes in adipose tissue homeostasis.

Key words: Adiponectin, adipose tissue homeostasis, hormone sensitive lipase

INTRODUCTION

Adipose tissue is an endocrine organ, and it acts as an energy reservoir such as storage of lipids when there is an excessive intake of fatty acids. Adipose tissue plays a key role in maintaining the lipid homeostasis. The core form of energy is stored in adipose tissue as triacylglycerol (TAG).[1] The formation of TG in adipose is in two ways 1. *De novo* lipogenesis form non-lipid metabolite 2. Increased uptake of fatty acid form circulation. Adipose tissue lipolysis is a catabolic process that is hydrolysis of TAG into fatty acids and glycerol. The TAG is break - down by three major hydrolytic enzymes such as lipase 1. Adipose triglyceride lipase (ATGL), Hormone-sensitive lipase (HSL), and monoacylglycerol lipase (MGL). The non-esterified fatty acids and glycerol are the final outcome of lipolysis. The fatty acids and glycerol are entering into the circulation for further use. The fatty acids in the circulation are involved in metabolism with a different organ such as liver, heart, and skeletal muscle. Some fatty acids are retained in the adipose tissue for its re-esterified into TG formation through glycerol-sn-3-phosphate acyltransferase. The glycerol is sustained in adipose tissue because some amount of glycerol kinase is needed for its metabolism. Normally per day 100-300 g of TAG is the mean turnover rate in human. The imbalance between these anabolism and catabolism leads to the metabolic disorder such as obesity and diabetes mellitus.[2]

Address for correspondence:
V. G. Abilash, School of Bioscience and Technology, VIT University, Vellore - 632 014, Tamil Nadu, India.
E-mail: abilash.vg@vit.ac.in

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The one mole of TAG hydrolyzed and forms 3 FA and 1 glycerol in the adipose tissue through two intermediates such as DAG and MAG.[3] Elevated expression of ATGL is found in adipose tissue which helps adipocyte differentiation but the HSL has privileged ability of hydrolyzing TAG.[4] HSL acts as a persistent rate limiting key enzyme for hydrolyzing DAG during lipolysis. In this article, we concern mainly on mutation in HSL and MGL (lipolysis) using sorting intolerant from tolerant (SIFT) and PolyPhen-2.

SIFT prediction is a bio-informatics tool depends on sequence alignments which is mediated through the degree of conservation of amino acid residues that are taken from closely related sequences, collected through PSI-BLAST.[5] The analysis of (SIFT) would be apply to naturally happening nonsynonymous polymorphisms or laboratory-mediated missense mutations.

Polymorphism phenotyping v2 (PolyPhen-2) is one of the tools which determine potential impact of an amino acid replacement on its structure and function of a protein. The Website of PolyPhen-2 interface could be http://genetics.bwh.harvard.edu/pph2/.

HSL activation plays an important role in rate limiting step in hydrolyzing DAG in adipose tissue.[6] HSL gene and protein structure are varying size from 88 to 130 kDa, generated by the same gene with different promoters in various tissue.[7-10] HSL in adipocyte is made up of catalytic domain in the position of N-terminal and C-terminal which is similar to all isoform of HSL. Amino acid residue such as Ser, Asp, and His present in catalytic domain and also it is phosphorylated site with its regulation. The HSL found to be hydrolysis DAG into MAG. In *in vitro* analysis, HSL has a more hydrolytic activity 10-fold with DAG than TAG. Sn-1 and Sn-3 positions of FA are highly prefered by HSL gene.[11] Cholesterol and retinyl esters are also catalyzed by HSL gene.[3] Catecholamines and insulin regulates the hormone HSL through cAMP activity. HSL is activated by phosphorylating it using PKA.[12] There are three amino acid site such as Ser 563, Ser 659, and Ser 660 are phosphorylated. Ser 660 is involved in kinase pathway and activate lipolysis pathway. The Ser 563 role for activation of HSL is still needs to be elucidated.[13,14]

MGL has an amino acid sequence containing of amino acids (302) with 33 kDa (molecular weight). Monoacylglycerols generated by HSL activation is finally hydrolyzed by MGL. MGL is continuously and abundantly uttered in AT and considered not a regulating enzyme in lipolysis. Monoacylglycerols further hydrolyzed into fatty acids and glycerol.[15] MGL catalysis the MAG in the position of 1 (3) and 2-esters bonds equally and is away form *in vitro* hydrolytic action against DAG, TAG or cholesteryl esters. It had been identified both the MGL structural element and its catalytic residues. Site-directed mutagenesis was utilized to identify and establish the residues. It has Asp 239, Ser 122 in a GXSXG motif, and His 269.[16]

Adipose tissue maintains its homeostasis by producing biologically active compound such as adipokines. Adiponectin is considered as fascinating adipokines. Adiponectin is a 29k-Da protein, which is constitutively expressed in adipose tissue. Adiponectin was mentioned in the beginning as ACRP30 - adipocyte complement-related protein of 30 kDa because it is similar to that of C1q (complement factor).[17] Adiponectin is highly connected with the insulin resistance, obesity, lipodystrophy, and other metabolic disorders.[18,19]

Adiponectin monomer is consists of three domains such as a variable N-terminal domain, several G–X–X repeats (a α-helical collagenous "stalk"), and a characteristic globular domain in the C-terminal (400 amino acids).[20] There are two receptors of adiponectin such as AdipoR1 and AdipoR2.[21] Adiponectin plays an important role in building insulin sensitivity, induce oxidation of fatty acids, attenuate the glucose metabolism through activating AMPK.[20]

MATERIALS AND METHODS

In Silico analysis

Data retrieval - single nucleotide polymorphism was recoup using database provided by National Center for Biotechnological Information. All the pathogenic single nucleotide polymorphisms (SNPs) were regained for HSL, MGL, and adiponectin gene which is involved in adipose tissue lysis and adipokines. NCBI server can be reached at (http://www.ncbi.nlm.nih.gov/snp/).

Estimation of missense mutation of SNPs using a sequence homology tool (SIFT)-SIFT prognosis is based on the degree of conservation of amino acid residues in sequence alignments derived from closely identical sequences, collected via PSI-BLAST. SIFT can be applied either to naturally occurring nonsynonymous polymorphisms or missense mutations induced in the laboratory. This procedure involves in multistep such as (1) looks for identical sequences, (2) chooses closely related sequences that may share identical function, (3) obtains multiple alignment of these chosen sequences, and (4) calculates normalized probabilities for all possible substitutions at each position from the alignment. Substitutions at each position with normalized probabilities less than a tolerance index of 0.05 are predicted to be in tolerantor deleterious; those more than or equal to 0.05 are predicted to be tolerated.[22]

Predicting effect of a single residue substitution on protein function using polyphen-2web server - polyphen-2 is a tool which determines possible impact of an amino acid substitution on the structure and function of a human protein. The PolyPhen-2Web interface can be reached at http://genetics.

bwh.harvard.edu/pph2/. The input form at this URL allows querying for a single individual amino acid substitution or a coding, nonsynonymous SNP annotated in the dbSNP database. The prediction is based on a number of sequence, phylogenetic, and structural features characterizing the substitution. For a given amino acid substitution in a protein, PolyPhen-2 extracts various sequence and structure-based features of the substitution site and feeds them to a probabilistic classifier.[23]

RESULTS

The SNP IDs with amino acid change that are associated with missense SNPs were submitted as input to SIFT and POLYPHEN servers.

SIFT analysis

At each amino acid position substitutions were normalized probabilities <a tolerance index of 0.05 are considered to be intolerant or deleterious; those >or = 0.05 are considered to be tolerated. Tables 1-3 show results for SIFT analysis.From tolerance index it can be concluded that SNPs of HSL and adiponectin genes show tolerance index <0 and hence are damaging. From Table 1, out of 18 SNPs: SIFT reveals 7 SNPs of HSL gene were found to be damaging with tolerance index up to 0. Table 2 predicted no risk of any damaging effect. However, from Table 3 and 8 SNPs were predicted to have a probable damaging effect by SIFT analysis.

PolyPhen-2 analysis

Polyphen server works with UNIPROT gene ID. Polyphen score of 0 indicates benign and values near to 1 are predicted to be probably or possibly damaging. Table 1 shows 5 SNPs were predicted to be probably damaging by POLYPHEN for HSL. Table 2 predicted no risk of any damaging effect. Tables 3, 8 SNPs were predicted to have a probable damaging effect by polyphen-2analysis.

DISCUSSION

HSL deficient mice shows the outcome of brown adipocyte which is identified by upregulation of UCP-1.[24] The dysregulation of HSL shows as the there is a highly defective lipolysis and decreases the adipose tissue mass. Inhibition of HSL by inhibitor decreases the plasma fatty acid level *in vivo*.[25] Treating of adipose tissue with the glucose and insulin results in elevated lipolysis and HSL level is maintained.[26] Inhibition of HSL results in accomplished non-appearance of discharge of FA from AT.[26,27]

In the rodent model, glucose acts as a positive regulator for HSL induced lipolysis. Decreased glucose level attenuates the activity of HSL mediated lipolysis.[28] The increased omnetal adipocyte catabolic capacity is due to constitutive expression level of HSL. A dysregulation in HSL defects catabolism of lipids in obese model. The decreased HSL level is determined in both the insulin resistant and Type 2 diabetes condition. The defect in HSL expression was determined in obese and lean individual preadipocyte culture *in vitro*. The adipocyte of the women with poly cystic ovarian syndrome shows defect in the expression of HSL protein.[29]

Finally, it is concluded that defect in HSL leads to disorders found metabolically such as obesity, resistance in insulin level, Type 2 diabetes, and poly cystic ovarian syndrome. Prevention of HSL defects using agonist would protect form metabolic disorders.

Adiponectin show defending activity in different processes such as energy metabolism, inflammation, and cell proliferation. Nigro *et al*., observed that adiponectin has essential role in therapies for the anticipation and/or for the management of obesity and obesity associated diseases such as antihyperglycemic, antiatherogenic, and anti-inflammatory properties.[30] Adiponectin has protective role in cardiovascular health also. In a recent review, it has been summarized that after understanding of role of adiponectin in cardiovascular health, it is useful for determination of

Table 1: SIFT and Polyphen analysis of SNPs associated with MGL gene

Parameters						SIFT		Polyphen	
SNP	Function	Nucleotide change	Amino acid change	Amino acid	mRNA position	Using homologues in the protein alignment		Score	Prediction
						Prediction	Score		
rs1804711	Missense	G/A	R202Q	R	792	Tolerated	0.34	0.005	Benign
				Q		Tolerated	0.09		
rs11538700	Missense	T/G	M288R	M	288	Tolerated	0.17	0.002	Benign
				R		Damaging	0.02		

[a]The dissimilarity in human genome contains a substitutions in single nucleotide, where one nucleotide change by one another (A,T,G,C), [b]Amino acid denoted with one letter symbol as per IUPAC IUB Commission on Biochemical Nomenclature, [c]The SIFT score ≤ 0.05 are determined by the algorithm to damaging or deleterious amino acid substitutions, whereas scores >0.05 are predicted as tolerant, [d]Polyphen score 0 indicates benign and values equal to 1 are considered as probably damaging. (a,b,c,d): The significant value for SIFT (P = 0.0593) and Polyphen (P = 0.1451) which is based on the damages or deleterious amino acid substitution for MGL gene

Table 2: SIFT and Polyphen analysis of SNPs associated with HSL gene

Parameters						SIFT		Polyphen	
SNP	Function	Nucleotide change	Amino acid change	Amino acid	mRNA position	Using homologues in the protein alignment		Score	Prediction
						Prediction	Score		
rs3745238	Missense	G/A	R217L	R	927	Tolerated	0.73	0.031	Benign
				L		Damaging	0.03		
rs3745238	Missense	G/A	R217Q	R	927	Tolerated	0.73	0	Benign
				Q		Tolerated	1		
rs7246232	Missense	C/A	R938S	R	938	Tolerated	1	0.014	Benign
				S		Tolerated	0.63		
rs16975748	Missense	S/T	S177T	S	806	Tolerated	1	0.009	Benign
				T		Tolerated	0.07		
rs16975750	Missense	T/C	Y100H	Y	575	Tolerated	0.19	0	Benign
				H		Tolerated	0.31		
rs33921216	Missense	A/C	N499H	N	1772	Tolerated	1	0.998	Probably damaging
				H		Tolerated	0.33		
rs34052647	Missense	C/T	R611C	R	2108	Tolerated	1	0.998	Probably damaging
				C		Damaging	0		
rs34080774	Missense	G/C	Q127H	Q	658	Tolerated	0.8	0.01	Benign
				H		Damaging	0.01		
rs34348028	Missense	C/T	P146S	P	713	Tolerated	1	0.006	Benign
				S		Damaging	0.02		
rs34996020	Missense	C/T	A194V	A	858	Tolerated	1	0.003	Benign
				V		Tolerated	0.71		
rs35938529	Missense	A/T	K497N	K	1768	Tolerated	0.98	0.998	Probably damaging
				N		Tolerated	0.29		
rs45603141	Missense	G/A	G742R	G	2501	Tolerated	1	0.827	Probably damaging
				R		Damaging	0		
rs61518179	Missense	C/T	S232F	S	972	Tolerated	1	0.006	Benign
				F		Damaging	0.04		
rs70937096	Missense	C/T	T277M	T	1107	Tolerated	1	0.004	Benign
				M		Tolerated	0.15		
rs70937098	Missense	G/T	R882S	R	2923	Tolerated	0.58	0.027	Benign
				S		Tolerated	0.29		
rs70937099	Missense	T/C	S888P	S	2939	Tolerated	0.75	0.008	Benign
				P		Tolerated	0.3		
rs112497256	Missense	G/A	R333Q	R	1275	Tolerated	1	0.475	Probably damaging
				Q		Damaging	0		
rs116152392	Missense	C/T	P99S	P	572	Tolerated	0.47	0.009	Benign
				S		Tolerated	0.35		
rs851303			Not found						

[a]The dissimilarity in human genome contains a substitutions in single nucleotide, where one nucleotide change by one another (A,T,G,C), [b]Amino acid denoted with one letter symbol as per IUPAC IUB Commission on Biochemical Nomenclature, [c]The SIFT score $\leq$ 0.05 are determined by the algorithm to damaging or deleterious amino acid substitutions, whereas scores >0.05 are predicted as tolerant, [d]Polyphen score 0 indicates benign and values equal to 1 are considered as probably damaging. (a,b,c,d): The significant value for SIFT (P = 0.0004) and Polyphen (P < 0.0001) which is based on the damages or deleterious amino acid substitution for HSL gene

Table 3: SIFT and Polyphen analysis of SNPs associated with adiponectin gene

Parameters						SIFT		Polyphen	
SNP	Function	Nucleotide change	Amino acid change	Amino acid	mRNA position	Using homologues in the protein alignment		Score	Prediction
						Prediction	Score		
rs13061862	Missense	G/T	G54V	G	296	Tolerated	1	1	Probably damaging
				V		Damaging	0		
rs17366743	Missense	T/C	Y111H	Y	466	Tolerated	1	0.012	Benign
				H		Tolerated	0.2		
rs62625753	Missense	G/A	G90S	G	403	Tolerated	1	1	Probably damaging
				S		Damaging	0		
rs72563731	Missense	C/T	A108V	A	458	Tolerated	1	0.608	Probably damaging
				V		Damaging	0		
rs78685763	Missense	G/A	R131H	R	527	Tolerated	1	0.892	Probably damaging
				H		Damaging	0		
rs79645624	Missense	G/T	R112L	R	470	Tolerated	1	0.771	Probably damaging
				L		Damaging	0		
rs79645624	Missense	G/C	R112P	R	470	Tolerated	1	0.959	Probably damaging
				P		Damaging	0		
rs113716447	Missense	C/T	A161V	A	617	Tolerated	0.21	0.257	Benign
				V		Damaging	0		
rs114155159	Missense	T/A	L9Q	L	161	Tolerated	1	0.871	Probably damaging
				Q		Tolerated	0.08		
rs121917815	Missense	C/T	R112C	R	469	Tolerated	1	0.947	Probably damaging
				C		Damaging	0		

[a]The dissimilarity in human genome contains a substitutions in single nucleotide, where one nucleotide change by one another (A,T,G,C), [b]Amino acid denoted with one letter symbol as per IUPAC IUB Commission on Biochemical Nomenclature, [c]The SIFT score ≤ 0.05 are determined by the algorithm to damaging or deleterious amino acid substitutions, whereas scores >0.05 are predicted as tolerant, [d]Polyphen score 0 indicates benign and values equal to 1 are considered as probably damaging. (a,b,c,d): The significant value for SIFT (P < 0.0001) and Polyphen (P = 0.0001) which is based on the damages or deleterious amino acid substitution for Adiponectin gene

receptor and post-receptor signaling process that induces the cardiovascular protective role of adiponectin. Discovery of adiponectin-targeted drug could be having important role in the treatment of obesity, diabetes and CVD.[31] In a previous study, it has been revealed that adiponectin has role in treating of vascular diseases.[32] The SNPs found with the risk of type 2 diabetes, obesity and diabetic nephropathy (DN) is +45G15G(T/G) in exon 2 and +276G/T in intron 2 of the AdipoQ gene. The SNPs found in the promoter region, inclusive -11426A/G, -11377C/G, and -11391G/A, are establish to be related with T2D and DN. Recent studies have observed that the polymorphisms in promoter region would be involving in the AdipoQ promoter activity.[33]

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