

Functional Protein Analysis of Hypertriglyceridemia: A Bioinformatic Approach

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Abstract

Hypertriglyceridemia is defined as an abnormal concentration of triglycerides in blood and is a commonly encountered lipid abnormality frequently associated with other lipid and metabolic derangements. Prolonged hypertriglyceridemia may produce neuropathological and abnormal metabolic changes particularly in peripheral sensory nerves. In the present study, we evaluated the role of several proteins that are likely to be involved in hypertriglyceridemia by employing multiple sequence alignment using ClustalW tool and constructed a phylogenetic tree using functional protein sequences extracted from NCBI. The phylogeny tree was constructed with Neighbor Joining Algorithm using bioinformatic principles and applications. The association of apolipoprotein C-II, proinsulin, fatty acid binding protein, sterol regulatory element binding transcription factor, angiotensin I converting enzyme, lipin 1, sterile co-A desaturase, cholesteryl ester transfer protein, and other apolipoproteins in hypertriglyceridemia suggests that a close interaction between these proteins may exist that may underlie the pathogenesis of hypertriglyceridemia. The results of the present bioinformatics study indicate a predominant involvement of apolipoprotein C-II, proinsulin in comparison to other proteins in the pathogenesis of hypertriglyceridemia.

Keywords: Hypertriglyceridemia; apolipoprotein C-II; proinsulin; bioinformatic analysis

Introduction

The two main sources of plasma triglycerides (also known as triacylglycerol) are exogenous (i.e., from dietary fat) and carried in chylomicrons, and endogenous (from the liver) and carried in very low-density lipoprotein (VLDL) particles. In capillaries within fat and muscle tissue, these lipoproteins and chylomicrons are hydrolyzed by lipoprotein lipase into free fatty acids. After a meal, over 90% of the circulating triglycerides originate in the intestine and are secreted in chylomicrons, whereas during periods of fasting, endogenous triglycerides secreted by the liver as VLDL predominate. The increase in plasma of triglyceride-rich proteins results from the liver and intestine (by means of upregulated synthetic and secondary pathways) or through decreased peripheral catabolism (mainly from reduced lipoprotein lipase activity) (George et al 2007).

Lipoproteins are macromolecular assemblies that contain lipids and proteins. The lipid constituents include free and esterified cholesterol, triglycerides and phospholipids. Apolipoproteins also known as apoproteins provide structural stability to the lipoproteins and also may function as ligands in lipoprotein receptor interactions or as cofactors in enzymatic processes that regulate lipoprotein metabolism. Table 1 (Goodman and Gilman 2006) describes apolipoprotein that have well defined roles in plasma lipoprotein metabolism. The lipoproteins were discovered by separation of the proteins after delipidation of plasma lipoproteins - initially classified as apolipoprotein A if present in alpha-lipoproteins (HDL), apolipoprotein B if present in beta-lipoproteins (LDL) and apolipoprotein C if present in pre-beta-lipoprotein (VLDL)

(Fredrickson 1974). Following further protein purification, sequencing, and genomic studies, these were reclassified and the major apolipoproteins affecting lipoprotein metabolism, apolipoproteins A1, A2, A4, B₁₀₀, B₄₈, C1, C2, C3 and E, were identified (Li and Chan 1999).

Diabetes mellitus association with hypertriglyceridemia

Diabetes mellitus is estimated to affect 6% of the population and DM2 accounts for 90–95% of diabetes cases (75). FFA act as incretins, that is, augment glucose stimulated insulin secretion, which is important under physiological conditions. However, FFA alone is not secretagogues. A two-arm signalling pathway is proposed to trigger secretion of insulin. The first arm is associated with acetyl-CoA mediated ATP/ADP ratio increase, which closes ATP-sensitive K⁺ channels, thereby depolarizing the cell. The consequence is the prolonged open-time of voltage-dependent Ca-channels. Finally the elevated intracellular Ca²⁺ concentration modulates kinases and other signalling proteins managing insulin secretion. The activity of the second arm is associated with pyruvate synthesis from glucose, followed by oxaloacetate and citrate production which is responsible for the subsequent malonyl-CoA synthesis. The latter is a switch repressing α -oxidation and stimulating synthesis of long-chain-CoA (LC-CoA) and complex lipids — diacylglycerols and phosphatidate. FFA is implicated by being a direct substrate for LC-CoA synthesis. Short term exposure to high FFA plasma concentrations has been proved to augment glucose-stimulated insulin secretion, while long-term

oversupply with FFA increases basal insulin secretion and exacerbates glucose-dependent secretion. Lewis GF et al (1991) re-

ported that moderate fasting hypertriglyceridemia in non insulin dependent diabetes mellitus predictive of a constellation of post-

Apolipoprotein	Average Concentration (mg/dL)	Chromosome	Molecular mass, KD	Sites of synthesis
ApoA-I	130	11	~29	Liver, Intestine.
ApoA-II	40	1	~17	Liver.
ApoA-V	<1	11	~40	Liver.
ApoB-100	85	2	~513	Liver.
ApoB-48	fluctuates according to dietary intake	2	~241	Intestine.
ApoC-I	6	19	~6.6	Liver.
ApoC-II	3	19	8.9	Liver.
ApoC-III	12	11	8.8	Liver.
ApoC-E	5	19	34	Liver, Brain, skin, gonads, spleen.

Table 1. Apolipoproteins.

prandial changes in lipids and lipoproteins that may potentiate the already unfavorable atherogenic fasting lipid profile.

Experimental Protocol and Results

The present research aims at finding the proteins responsible for hypertriglyceridemia in two phases. The first phase of the research attempts to identify the candidate proteins that cause hypertriglyceridemia. The data pertaining to these proteins is extracted from the databases that are available online for free access. The functional protein sequences of these proteins in FASTA are to be extracted from (National Center for Biotechnology Information (NCBI), (<http://www.ncbi.nlm.nih.gov>).

The second phase of the research analyzes the data by employing Multiple Sequence Alignment using ClustalW online tool. These alignments produce a Phylogenetic Tree along with the alignment scores. From the tree the results of the research are to be inferred in the last phase of the research.

ClustalW, a web based progressive alignment tool for Multiple Sequence Alignment (MSA). ClustalW adds sequences one by one to the existing alignment to build a new alignment because of its progressive nature. Progressive in this context means, it starts with using pair wise method to determine the most related sequences and then progressively adding less related sequences initial alignment. The order of the sequences to be added to the new alignment is indicated by a precomputed phylogenetic tree called a guide tree. The guide tree is constructed using the similarity of all possible pairs of sequences. The functional protein sequences of 16 proteins that are believed to be involved in the pathogenesis of diabetic neuropathy collected from NCBI (National Center for Biotechnology Information <http://www.ncbi.nlm.nih.gov>) in FASTA forms (these sequences are given

to clustalw <http://www.ebi.ac.uk/clustalw>) were analyzed for the multiple sequence alignment (it calculates that the best match for the selected sequences, and lines them up so that the identities, similarities and differences can be seen) and the resultant score the phylogeny tree constructed based on these results are given in Table 2 and Figure 1 respectively. The phylogeny shows the distance between the protein sequences. The protein sequences with minimum distance are apolipoprotein C-II and proinsulin, which suggests that these proteins play a significant role in the pathogenesis of hypertriglyceridemia.

Discussion

Hypertriglyceridemia is common in Indians. However, the exact cause for this high incidence has never been adequately explained. Although Indians as a race may have a higher risk to develop various features of metabolic syndrome, what are these genetic factor(s) has never been elucidated. One suggestion that has been made is the thrifty gene hypothesis. It was postulated the existence of metabolically thrifty genes that permit efficient utilization of food leading to fat deposition and weight gain at times of food abundance making the gene-bearer better able to survive during times of famine. Examples of thrifty genes included insulin and leptin. Nondiabetic Nauruans and Arizona. Pima Indians have postprandial levels of plasma proinsulin that are almost triple those of Europeans. These populations when given ample food first develop hypertriglyceridemia and then develop obesity, a propensity that they exhibit more compared to Europeans. Experimental rats carrying genes predisposing them to hypertriglyceridemia and obesity survive starvation better than do normal rats. In addition to the genetic component, hypertriglyceridemia and metabolic syndrome also involves environmental and lifestyle risk factors in the form of high calorie intake and low exercise (figure 2).

Protein symbol	Protein name & Protein length	Location	Function
APOA5	Apolipoprotein A-V 366 aa	It is located proximal to the apolipoprotein gene cluster on chromosome 11q23.	The protein encoded by this gene is an apolipoprotein and an important determinant of plasma triglyceride levels, a major risk factor for coronary artery disease. It is a component of high density lipoprotein
RP1	Retinitis pigmentosa 2156aa	Chromosome: 8 Location: 8q11-13	
LIPI	Lipase,member1 460 aa	Chromosome: 21 Location: 21q11.2	Lipid catabolism
APOA1	Apolipoprotein A1 267 aa	Chromosome: 11; Location: 11q23-q24	Which is the major protein component of high-density lipoprotein (HDL) in plasma. The protein promotes cholesterol efflux from tissues to the liver for excretion, and it is a cofactor for lecithin cholesterol acyl transferase (LCAT), which is responsible for the formation of most plasma cholesteryl esters.
APOC3	Apolipoprotein C-III 99 aa	Chromosome: 11; Location: 11q23.1-23.2	APOC3 inhibits lipoprotein lipase and hepatic lipase; it is thought to delay catabolism of triglyceride-rich particles. The APOA1, APOC3 and APOA4 genes are closely linked in both rat and human genomes. The A-I and A-IV genes are transcribed from the same strand, while the A-1 and C-III genes are convergent transcribed. An increase in apoC-III levels induces the development of hypertriglyceridemia.
LPL	Lipoprotein lipase 475 aa	Chromosome:8 Location: 8P22	LPL encodes lipoprotein lipase, which is expressed in heart, muscle, and adipose tissue. LPL functions as a homodimer, and has the dual functions of triglyceride hydrolase and ligand/bridging factor for receptor-mediated lipoprotein uptake. Severe mutations that cause LPL deficiency result in type I hyperlipoproteinemia, while less extreme mutations in LPL are linked to many disorders of lipoprotein metabolism.
APOE	Apolipoprotein E 317 aa	Chromosome: 19 Location: 19q13.	This is a main apoprotein of the chylomicron, binds to a specific receptor on liver cells and peripheral cells. ApoE is essential for the normal catabolism of triglyceride-rich lipoprotein constituents.

INS	Proinsulin 110 aa	Chromosome: 11 Location: 11p15.5	Promotes lipogenesis
APOC2	Apolipoprotein C-II 101 aa	Chromosome: 19 Location: 19q13.2	The protein encoded by this gene is secreted in plasma where it is a component of very low-density lipoprotein. This protein activates the enzyme lipoprotein lipase, which hydrolyzes triglycerides and thus provides free fatty acids for cells. Mutations in this gene cause hyperlipoproteinemia type IB
FABP1	Fattyacid binding protein 2 132 aa	Chromosome: 4 Location: 4q28-q31	These are thought to participate in the uptake, intracellular metabolism and/or transport of long-chain fatty acids. They may also be responsible in the modulation of cell growth and proliferation
SREBF1	Sterol regulatory element binding transcription factor 1 1147 aa	Chromosome: 17 Location: 17p11.2	This gene encodes a transcription factor that binds to the sterol regulatory element-1 (SRE1), which is a decamer flanking the low-density lipoprotein receptor gene and some genes involved in sterol biosynthesis. The protein is synthesized as a precursor that is attached to the nuclear membrane and endoplasmic reticulum.
SLC10A2	Solute carrier family 10 506 aa	Chromosome: 13 Location: 13q33	Facilitates the entero-hepatic circulation of the of bile salts and play s a key role in cholesterol metabolism
ACE	Angiotensin I converting enzyme 732 aa		
LPLA2	Lipin 1 146 aa	Chromosome: 2 Location: 2p25.1	This gene represents a candidate gene for human lipodystrophy, characterized by loss of body fat, fatty liver, hypertriglyceridemia, and insulin resistance
SCD	Stearoyl-coA desaturase 359 aa	Chromosome: 10 Location: 10q23-q24	It is an iron-containing enzyme that catalyzes a rate-limiting step in the synthesis of unsaturated fatty acids. The principal product of SCD is oleic acid, which is formed by desaturation of stearic acid.
CETP	Cholesteryl ester transfer protein 493 aa	Chromosome: 16 Location: 16q21	This protein transfers cholesteryl esters between lipoproteins. It may affect susceptibility to arteriosclerosis.

Table 2: Table shows the genes/proteins that has been studied in the present study that are believed to be involved in hypertriglyceridemia.

Phylogram

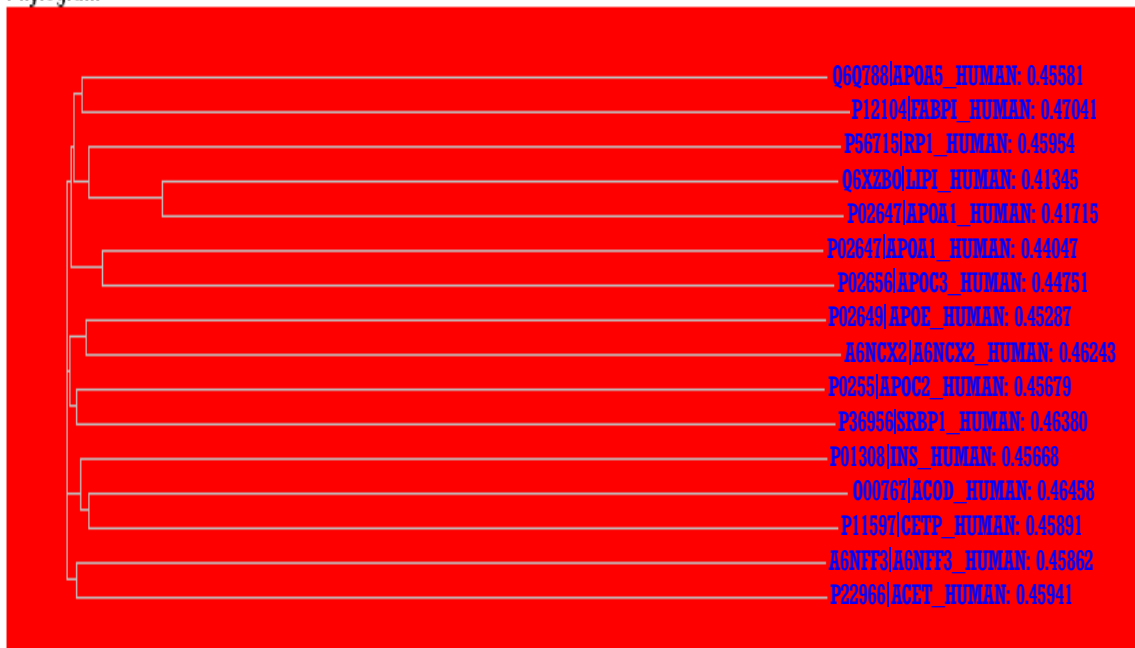


Figure 1: The phylogenetic tree constructed based on alignment scores of all the protein sequences involved in hypertriglyceridemia. high degree of homology was noted for apolipoprotein C-II and proinsulin.

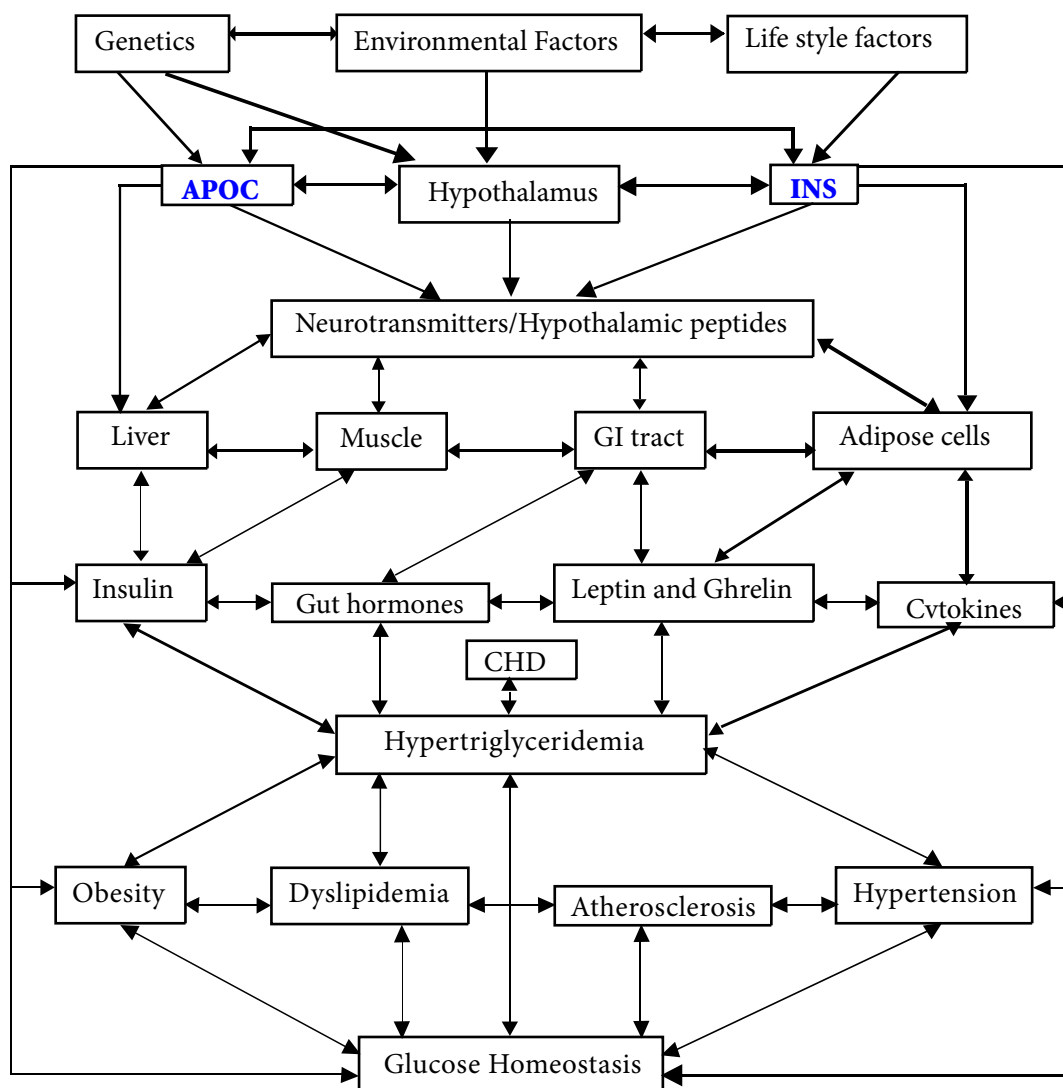


Figure 2: Scheme showing relationship between interaction(s) between various genetic and environmental factors and target organs involved in the development of hypertriglyceridemia.

Recently Jogi Raju et al (2007) reported the hyper filtration with metabolic syndrome in relation to hypertriglyceridemia. Apolipoprotein A5 (APOA5) is a newly described member of the apolipoprotein gene family whose initial discovery arose from comparative sequence analysis of the mammalian APOA1/C3/A4 gene cluster. Studies in humans have suggested an important role for APOA5 in determining plasma triglyceride concentrations (Len A. Pennacchio; Edward M. Rubin, 2003). In these experiments, polymorphisms in the human gene were found to define several common haplotypes that were associated with significant changes in triglyceride concentrations in multiple populations. Tsutomu M and Masa-aki M (2005) have prioritized the significance of 6131 well-annotated human genes in terms of the distance on the plane from the centroid of 'metabolic syndrome'-related genes distribution according to them a methodology to search for genes associated with multifactorial diseases by integrating the large amount of accumulated knowledge is seriously needed.

Proinsulin has anti-inflammatory actions. Proinsulin suppresses the production of TNF-alpha, IL-6, IL-1, IL-2, and macrophage migration inhibitory factor (MIF), which are pro-inflammatory molecules and enhances the production of IL-4 and IL-10 that are anti-inflammatory cytokines. This suggests that the presence and purpose of hyperinsulinemia in normal Indians is to prevent or abrogate the low-grade systemic inflammation that is inherent in them as evidenced by elevated levels of CRP, and possibly, TNF-alpha and IL-6. On the other hand, leptin has pro-inflammatory actions. Since hyperinsulinemia and hypertriglyceridemia are evident in Indian children compared white children, it is clear that features of low-grade systemic inflammation and metabolic syndrome are initiated very early in life.

Conclusion

Bioinformatics analysis of functional protein sequences of genes and related proteins that are involved in hypertriglyceridemia revealed a high degree of homology between apolipoprotein C-II

and proinsulin. It is evident from the preceding discussion and results of the present bioinformatics study that apolipoprotein C-II and proinsulin play a significant role in the pathobiology of hypertriglyceridemia.

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