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Is Apolipoprotein A5 -1131t>C Polymorpshism A Contributing Factor For Tryglyceride Level In Metabolic Syndrome?

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Abstract

Metabolic syndrome is a group of metabolic disorders with high prevalence in several populations and is a risk factor for cardiovascular disease and stroke with high mortality rate. Dyslipidemia in metabolic syndrome is marked by the increase of triglycerides. Apolipoprotein A5 (ApoA5) regulates triglycerides by lowering production of VLDL (Very Low Density Lipoprotein) and increases LPL (Lipoprotein Lipase) activity. Apolipoprotein A5 -1131T>C polymorphism in the promoter region is linked to the increase of triglycerides in metabolic syndrome. This study aims to find the relationship of apolipoprotein A5 -1131T>C polymorphism with triglycerides level in metabolic syndrome. This study was a case control study involving 50 subjects with 25 metabolic syndrome subjects and 25 non-metabolic syndrome subjects. ApoA5 -1131T>C polymorphism was visualized with 5% agarose gel after restriction length fragment polymorphism (RFLP) digested with MseI. Data obtained were analyzed with unpaired t-test. Unpaired t-test result showed significant difference between ApoA5 -1131T>C polymorphism triglyceride level in metabolic syndrome and non-metabolic syndrome patient, be it TT or CC genotype with $p<0.05$. The TT genotype is found higher in metabolic syndrome compared to non-metabolic syndrome patients (72% vs 32%), CC genotype is found lesser in metabolic syndrome compared to non-metabolic syndrome (28% vs 68%), while heterozygous TC was not found in the subjects. This study showed that ApoA5 -1131T>C polymorphism was not a contributing factor for triglycerides level in metabolic syndrome. Increase in triglycerides in metabolic syndrome may be influenced by other ApoA5 SNPs.

Keywords: metabolic syndrome, ApoA5, ApoA5 -1131T>C, triglyceride, VLDL, LPL.

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1. Background

Metabolic syndrome is a group of metabolic disorders often linked with high mortality diseases like coronary heart disease and stroke (Cameron *et al*, 2004). Based on National Cholesterol Education Program Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults Treatment Panel II (NCEP ATP III), metabolic syndrome is a group of metabolic disorders, lipid or non-lipid, which fulfills 3 of 5 criteria such as central obesity, atherogenic dyslipidemia, hypertension, and abnormal plasma glucose level.

Metabolic syndrome prevalence varies between countries. According to NCEP ATP III, metabolic syndrome is a metabolic disorder with high prevalence. Metabolic syndrome is found in almost a quarter of adult US citizen (47 million individuals) or around 24% of population (Yamada *et al*, 2005), with Mexico having the highest prevalence (26.6% in ages between 20-69 years) (Cameron *et al*, 2004). Metabolic syndrome prevalence increases in Korea during the last decade with 31.3% in 2007 (Song *et al*, 2012).

Dyslipidemia in metabolic syndrome results in increase in serum triglycerides and decrease of serum high density lipoprotein (HDL). Lipoprotein Lipase (LPL), apolipoprotein A5 (ApoA5), amdapolipoprotein E (ApoE) affects triglycerides metabolism. LPL is the main enzyme responsible is triglyceride hydrolysis, ApoA5 regulates the level of triglycerides via lipolysis efficiency, and ApoE plays an important role in receptor mediated remnant clearance (Ariza*et al*, 2010).

Song *et al* (2012) stated that there was a significant relationship between ApoA5 -1131T>C polymorphism with triglyceride and HDL level as a risk factor in metabolic syndrome in the Korean population.

The study also stated identification of the C allele of ApoA5 -1131T>C polymorphism can help prevent metabolic syndrome. Yamada *et al* (2007) showed a significant relationship between ApoA5 -1131T>C polymorphism with increase of triglyceride and cholesterol levels in the Japanese population. The same observation also seen in a study by Grallert*et al* (2007) in the Caucasian population, ApoA5 -1131T>C polymorphism in the promoter region contributes to increase in triglycerides level in metabolic syndrome.

Method

This case control study involves 50 subjects with 25 metabolic syndrome subjects and 25 non-metabolic syndrome subjects with matching sex and age groups (Kusuma, 2012).

National Cholesterol Education Program Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults Treatment Panel III (NCEP ATP III) is used to determine the metabolic syndrome criteria.

ApoA5 polymorphism genotyping is done by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) using 5´-CCCCAGGAACTGGAGCGAAATT-3´(Forward) and 5´-TTCAAGCAGAGGGAAGCCTGTA-3´ (Reverse). The 50 µl PCR reaction was done on ice with 1 U Go Taq® Green Master Mix, 0.2 mM primers, *nuclease free water*, 200 µMdNTP, 5 µlbuffer (500 mMKCl, 14 mM MgCl2, 10 mMTris-HCl, pH 9.0). The reaction was done in thermal cycler preheated to 95^0C. The initial denaturation was done at 96^0C for 300 secs, followed by denaturation at 95^0C for 30 secs, annealing at 55^0C for 30 secs, extension at 72^0C for 30 secs, and final extension at 72^0C for 300 secs for 32 cycles. The 396bp amplicons were then visualized with 5% agarose gel. Amplicon digestion was then done using MseI restriction enzyme incubated at 65^0C for 2 hours.

The digested DNA (TT: 20bp, 105bp, 271bp; TC: 20bp, 105bp, 271bp, 291bp; CC: 105bp, 291bp) was then visualized using 5% agarose gel using gel documentation system.

All the data were obtained, clustered and statistically analyzed using unpaired t-test. Allelic frequency was determined by counting alleles and sample proportion. Hardy-Weinberg Equilibrium was used to assess the polymorphism.

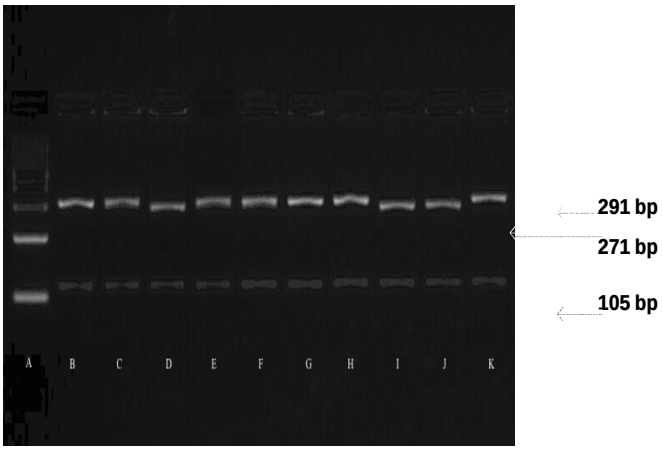
Results

This study was done on 25 metabolic syndrome subjects and 25 non-metabolic syndrome subjects. The subjects' triglycerides level is shown in table 1. The number of metabolic syndrome subjects with triglycerides level >150mg/dL (hypertriglyceridemia) were almost equal in numbers with the subjects with triglycerides level <150mg/dL (normotriglyceridemia). In non-metabolic syndrome group, the numbers of normotriglyceridemia subjects were higher compared to subjects with hypertriglyceridemia.

Table 1. Triglyceride levels for Metabolic Syndrome and Non-Metabolic Syndrome groups

		Metabolic Syndrome N (%)	Non Metabolic Syndrome N (%)
Triglyceride Level (mg/dL)	>150mg/dL (hipertrigliseridemia)	12 (48%)	1 (4%)
	<150mg/dL (normotriglisеridemia)	13 (52%)	24 (96%)
Total		25 (100%)	25 (100%)

PCR amplification showed a 369bp amplicon and after digestion with MseI, homozygous TT showed band at 271bp, 105bp, and 20bp (Picture 1, the 20bp band is not visible in the picture).



Picture 1: RFLP gel image for ApoA5 -1131T>C. Lane A: Marker; Lane B,C,E,F, G,H,K: homozygous CC; Lane: D,I, J: homozygous TT. 20bp band is not visible in the picture

In table 2, CC genotype was found higher in normotriglyceridemia compared to hypertriglyceridemia subjects. TT genotype (wildtype) was found higher in metabolic syndrome and CC genotype (homozygous variant) is found higher in non-metabolic syndrome subjects. The heterozygous TC genotype was not found in both the metabolic syndrome and non-metabolic syndrome groups (Table 3).

Table 2.Apolipoprotein A5 -1131T>C polymorphism in Hipertrigliseridemia and Normotrigliseridemia

		TT N (%)	CC N (%)
Triglyceride Level (mg/dL)	>150mg/dL (hipertrigliseridemia)	7 (10%)	6 (10%)
	<150mg/dL (normotrigliseidemia)	19 (30%)	18 (30%)

Apolipoprotein A5 -1131T>C polymorphism differs significantly in metabolic syndrome and non-metabolic syndrome groups ($p<0.05$) and a significant difference between Apolipoprotein A5 -1131T>C polymorphism with triglycerides level in metabolic syndrome and non-metabolic syndrome groups ($p<0.05$), for both TT and CC genotypes.

Table 3. ApolipoproteinA5 -1131 T>C genotype frequencyand TriglyserideLevel in Metabolic Syndrome and Non-Metabolic Syndrome

	ApoliproteinA5-1131T>C			
	TT		**CC**	
	Metabolic Syndrome (n=18)	**Non Metabolic Syndrome (n=8)**	**Metabolic Syndrome (n=7)**	**Non Metabolic Syndrome (n=17)**
Triglyceride Level (Mean± SD)	127,39 ± 45,35	84,63 ± 38,06	165,14 ± 53,90	94,24 ± 26,34
CI 95%	6,33-79,19		20,7-121,09	
p	0,029		0,012	

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In table 4, C allele frequency is found highest in non-metabolic syndrome subjects with 0.68 and T allele is found highest in metabolic syndrome subjects with 0.72. According to Hardy-Weinberg Equilibrium, the Apolipoprotein A5 -1131T>C polymorphism is in disequilibrium.

Table 4. Genotype and Allelic Frequencyof ApolipoproteinA5 -1131T>C Polymorphism In Metabolic Syndrome and Non-Metabolic Syndrome

		Apolipoprotein A5 Polymorphism					
		Genotype			*Allele Frequency*		*p*
		TT	**TC**	**CC**	**T**	**C**	
Metabolic Syndrome	***Observed***	18	0	7	72%	28%	<0,05
	Expected HW Frequency	12,96	10,08	1,96			
		51,84%	40,32%	7,84%			
Non Metabolic Syndrome	***Observed***	8	0	17	32%	68%	<0,05
	Expected HW Frequency	2,56	10,88	11,56			
		10,24%	43,52%	46,24%			

Discussion

In this study, TT genotype (wildtype) was found higher in the metabolic syndrome group, CC genotype was found higher in non-metabolic syndrome, and heterozygous TC genotype was not found in both groups. CC genotype was also found higher in normotriglyceridemia compared to hypertriglyceridemia. This observation was not similar to the studies done by Maasz*et al* (2007), Kim *et al* (2013), Zaki*et al* (2014), and Samadikuchaksarei*et al* (2011) which stated that CC genotype is found higher in hypertriglyceridemia compared to normotriglyceridemia.

The C allele frequency was higher and is linked with elevated triglycerides level, making it a risk allele for metabolic syndrome (Zaki*et al*, 2013). C allele frequency in this study was 0.28 in the metabolic syndrome group and 0.68 in the non-metabolic syndrome group. The C allele frequency was almost three times higher in non-metabolic syndrome compared to the metabolic syndrome group.

This observation is the opposite of a study done by Maasz*et al* (2007) where C allele in the metabolic syndrome group is higher compared to the non-metabolic syndrome group. A study in Korea by Song *et al* (2013) showed that the C allele frequency in higher in metabolic syndrome subjects. Haqparast*et al.* and Samadikuchaksaraei*et al.*in 2011 studied the Iranian population and found that C allele frequency is higher in the metabolic syndrome subjects compared to the non-metabolic syndrome subjects and the C allele contributes to significant increase of hypertriglyceridemia.

Apolipoprotein polymorphisms in ApoA1, ApoC3, ApoA4, and ApoA5 were linked to the increase of triglycerides level in previous studies. ApoA5 gene variants can influence ApoC3 in triglyceride metabolism. ApoC3 plays a role in increase in triglycerides level by repressing LPL and influenced triglycerides uptake by ApoE (Povel*et al*, 2011). So far, 5 known ApoA5 polymorphism are grouped into 3 haplotypes, ApoA5*1, ApoA5*2, and ApoA5*3 ApoA5*2 haplotype consists of -1131T>C, c.-3A>G, IVS3+476G>T and c.1259T>C polymorphisms while ApoA*3 haplotype consists of c.56G>C (S19W). ApoA5 polymorphism can affect protein transcription, which will influence LPL and leads to increase in triglycerides level (Maasz*et al*, 2007). The ApoA5 -1131T>C is known to cause disturbance in Peroxisome Proliferator Response Element (PPRE) affinity, which is regulated by Proliferator-Activated Receptor-α *(*PPAR-α) or by disturbing the binding with other regulators leading to lower ApoA5 gene expression (Bi *et al*, 2004).

The transcription factor that recognizes the binding site which leads to lower ApoA5 gene expression is still not known and believe to experience linkage disequilibrium with other location to increase triglyceride level (Talmud *et al*, 2002; Song *et al*, 2013). Other than ApoA5 polymorphism, increase in triglyceride level is also influenced by ApoE (Ariza*et al*, 2010). ApoE plays a role in higher triglycerides level due to its function to form lipoprotein-triglycerides, a high affinity ligand for LDL receptor and also functions in remnant lipoprotein catabolism. Sousa *et al* (2008) and Novotny *et al* (2014) stated that higher triglycerides level is seen in subject possessing ApoA5 and ApoE polymorphisms.

Previous study stated that there is a linkage disequilibrium between ApoC3/A4/A5 locus, ApoA5 -1131T>C, and S19W variants (Talmud *et al*, 2002). Niculescu*et al* (2010) stated that ApoA5 is not the only risk factor for metabolic syndrome by increasing triglycerides level. Haplotype mutation c.c.-3A>G (S19W) and strong linkage disequilibriumbetween ApoA5 with ApoC3 (-482C>T, and -455T>C) may also play an important role.

In this study, the C allele frequency is higher in non-metabolic syndrome group (0.68 vs. 0.28) and higher in the normotriglyceridemia (0.49 vs 0.46). This might be possible since ApoA5 -1131T>C polymorphism might not be the only factor that contributes to increase in triglycerides since there are 4 other known polymorphisms of the ApoA5 that might be linked to increase in triglycerides level (c.-3A>G, IVS3+476G>T, c.1259T>C, and c.56G>C (S19W) (Chandak*et al*, 2006). Other than the polymorphisms, serum triglycerides level can also be influenced by environmental factors such as nutrition, alcohol consumption, and smoking. This might be the reason that C allele does not have a direct influence in triglycerides level in metabolic syndrome group in this study. Body Mass Index (BMI) may also contribute to increase in triglycerides level via ApoA5 -1131T>C polymorphism.

A study by Zhu *et al* (2014), C allele frequency with increase in triglycerides is found in more obese subjects compared to the non-obese counterpart. This showed that people with normal BMI possessing ApoA5 -1131T>C polymorphism may have normal triglycerides level. BMI also contributes to the increase in triglycerides in people having ApoA5 -1131T>C polymorphism.

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