

## Association study of Apolipoprotein A5 gene (APOA5 gene) variant with the metabolic syndrome in local Pakistani population

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### Abstract

**Objective:** To explore the association of rs662799 variants of Apolipoprotein A5 gene with metabolic syndrome in Pakistani population.

**Methods:** The case-control study was conducted at Pakistan Institute of Medical Sciences, Islamabad, Pakistan from 2014 to 2016, and comprised subjects enrolled from the out-patient clinics. Groups were formed on the basis of preliminary screening for risk factors like obesity, insulin resistance, hypertension, dyslipidemia and fasting blood glucose levels. MetS was diagnosed based on the international diabetes federation criteria. Blood samples were collected for biochemical testing and deoxyribonucleic acid extraction. Genotyping of rs662799 was performed at the Genome Research Centre of the University of Hong Kong using Sequenom Mass ARRAY, iPLEX Gold technology. Data was analysed using SPSS 16 and Plink software.

**Results:** There were 712 subjects in two groups of 356 (50%) each. The overall mean age was 41.59±7.18 years. There was a significant association of risk allele C of rs662799 with metabolic syndrome (p=0.002). The risk showed strong association with dyslipidaemia (p=0.03) and obesity (p=0.01) which are risk phenotypes of metabolic syndrome in age- and gender-adjusted model.

**Conclusion:** The association of risk allele C of genetic variant rs662799 of Apolipoprotein A5 gene with dyslipidaemia and obesity may lead to the development of metabolic syndrome in the Pakistan adult population.

**Keywords:** Metabolic syndrome, APOA5, rs662799, CVD, Dyslipidaemia, T2DM. (JPMA 69: 301; 2019)

### Introduction

Metabolic syndrome (MetS) is a combination of complex risk factors, including central obesity, hypertension (HTN), dysglycaemia, and dyslipidaemia (DLP).<sup>1</sup> Its prevalence in Pakistan and worldwide is increasing.<sup>2,3</sup> If MetS persists, its risk factors, such as DLP, HTN and abdominal obesity, could lead to the development of cardiovascular diseases (CVDs) and type 2 diabetes mellitus (T2DM).<sup>4</sup> Previously, unhealthy sedentary lifestyle factors, such as alcohol drinking, lack of exercise and smoking, were considered additional risk for the development of MetS.<sup>5</sup> It has now been shown that genetic factors along with environmental risks are involved in the development of the MetS.<sup>6</sup>

On a molecular basis, MetS is considered a polygenic and multifactorial disorder due to the interaction of a number of different genes with environmental factors. Genes leading to significant disease susceptibility have

been identified in different ethnic groups worldwide like Apolipoprotein A5 (APOA5),<sup>7</sup> Leptin (LEP), Lipoprotein lipase (LPL), and Cholesteryl ester transfer protein (CETP) genes.<sup>8</sup> Among different candidate genes, APOA5 has shown significant association with MetS in different ethnic groups.<sup>7</sup> The APOA5 gene belongs to the apolipoprotein family of genes, located on chromosome 11q23 close to the APOA1-C3-A4 gene cluster. The gene consists of 4 exons coding for a 366 amino acid protein that determines plasma triglycerides (TG) levels.<sup>9</sup> The APOA5 gene product, APOA5 protein, is bound to TG-rich, high-density lipoproteins (HDL) responsible for lowering plasma TG levels by inhibiting very low-density lipoprotein (VLDL)-TG production and stimulating LPL-mediated TG hydrolysis. The LPL-mediated lipoprotein lipid hydrolysis releases free fatty acids under insulin stimulation, leading to the development of insulin resistance (IR).<sup>10</sup> Several single nucleotide polymorphisms (SNPs) in APOA5 gene have been identified through genome-wide association studies (GWAS) to be associated with cardio-metabolic disease traits, especially hypertriglyceridaemia (HTG).<sup>11</sup> Among these SNPs, rs662799 was found to be strongly associated with HTG and reduced HDL<sup>12</sup> and MetS in

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Hong Kong and Guangzhou Chinese,<sup>13</sup> Korean,<sup>7</sup> Japanese<sup>14</sup> and Caucasian populations.<sup>15</sup>

Given its role in TG metabolism and interaction with insulin, the current study was planned to explore SNP rs662799 in APOA5 for its association with MetS and its components in a population of Pakistani origin.

## Patients and Methods

The case-control study was conducted at Pakistan Institute of Medical Sciences, Islamabad, Pakistan, from 2014 to 2016. Approval was obtained from the ethics committees of Pir Mehr Ali Shah (PMAS) Arid Agriculture University, Rawalpindi, and Shaheed Zulfiqar Ali Bhutto Medical University (SZABMU), Islamabad. Written informed consent was obtained from all the subjects. Case and control groups were formed on the basis of preliminary screening for risk factors, including obesity, IR, HTN, DLP and fasting blood glucose (FBG) levels. Those already having T2DM, cancer, liver disease or who were hypertensives were excluded. The subjects were recruited from Pathology out-patients department (OPD) of SZABMU. Anthropometric measurements, like height (inches) and waist circumference (cm), were measured using a non-elastic measuring tape, while body weight (kg) was recorded on a weighing machine without shoes or heavy clothing. Body mass index (BMI) was calculated using body weight and height (Weight (Kg)/Height [Meters<sup>2</sup>]). Blood pressure (BP) was measured from the right arm while the subjects were in the sitting position. For biochemical assays and genotyping, 4 to 5 ml samples of venous blood were drawn and aliquoted in serum collection and ethylenediaminetetraacetic acid (EDTA)-coated tubes. An experienced medical officer performed all the relevant measurements and recordings. MetS was defined according to the criteria set out by the International Diabetes Federation (IDF).<sup>16</sup>

For biochemical lipid profile and FBG tests, serum collection tubes with whole blood were centrifuged at 3500rpm for 20 minutes. All assays were performed using standard kits with a Microlab 300 (Merck) spectrophotometer.

Tagging SNP rs662799, located in the promoter region of the APOA5 gene, was selected on the criteria of having minor allele frequency (MAF)  $\leq 0.05$  and odds ratio (OR)  $\geq 0.8$  from the Hapmapdata.<sup>17</sup> Deoxyribonucleic acid (DNA) was extracted using a modified organic method.<sup>18</sup> The extracted DNA was quantified on Nanodrop 2000 (Thermo Scientific). For SNP genotyping, polymerase chain reaction (PCR) and extension primers were designed using the Sequenom software. Genotyping was performed on Sequenom Mass ARRAY system (Sequenom, San Diego, CA, USA) with the iPLEX assay in the Genome Research Centre (GRC) of the University of Hong Kong, Hong Kong.

Means and standard deviation (SD) were calculated for all descriptive characteristics. Differences between cases and controls in anthropometric and biochemical variables were assessed using t-test. Genotype/allele frequencies were estimated and deviations from Hardy-Weinberg equilibrium were calculated using the Chi-square goodness-of-fit test. Logistic regression modelling was applied to assess gene-disease associations after adjustment for age and gender. OR and confidence intervals (CI) at 95% significance level were calculated.  $P < 0.05$  was considered statistically significant. Statistical analysis was performed using SPSS 16 and Plink (1.0.6).<sup>19</sup>

## Results

There were 712 subjects in two groups of 356(50%) each. The overall mean age was  $41.59 \pm 7.18$  years. There were 288(40.44%) females and 424(59.55%) males.

**Table-1:** Characteristics of study subjects.

Parameter	Units	Total (N=712)	Control (N=356)	Cases (N=356)	P Value
Age	Yrs	41.59±7.18	40.56±6.72	42.05±6.29	<.0001
SBP	mmHg	123.02±14.04	115.84±9.6	130.25±14.26	<.0001
DBP	mmHg	83.49±10.57	78.40±7.60	88.88±11.12	<.0001
Weight	Kg	70.72±9.29	66.35±6.09	75.10±9.87	<.0001
Waist	cm	90.90±9.70	82.50±3.90	99.40±5.50	<.0001
BMI	-	28.3±5.2	23.664±2.43	27.981±4.75	<.0001
Chol	mg/dl	192.34±44.17	184.17±40.1	200.56±46.28	<.0001
TG	mg/dl	172.06±91.40	122.36±20.5	220.15±90.77	<.0001
HDL	mg/dl	41.34±8.93	45.10±6.24	37.57±9.37	<.0001
LDL	mg/dl	116.52±41.25	114.59±38.8	118.43±43.02	<.0001
FBG	mg/dl	21.06±63.81	92.27±10.7	147.36±77.86	<.0001

SBP- Systolic Blood Pressure, DBP- Diastolic Blood Pressure, HDL-High Density Lipoprotein, LDL-Low Density Lipoprotein, FBG- Fasting blood glucose, Chol-Cholesterol, TG-Triglyceride.

**Table-2:** Distribution of genotypic and risk allele frequency of rs662799.

Subjects	Genotypic frequency (%)			P Value	Risk Allele (C) Frequency (%)
	TT	TC	CC		
Controls	73.5	25	1.1	0.51	14
Cases	63.9	31.8	2.7	0.59	19
Male	70.0	26.4	2.5	0.99	16
Female	66.7	31.5	1.2	0.23	17

**Table-3:** Odds Ratio of MetS in Carriers of the Minor Allele C of rs662799 SNP.

Subjects	MAF	TT/TC/CC	Odds Ratio (CI 95%)	P Value
Total	MetS 0.331	224/116/10	1.5 (1.55-1.58)	0.002
	Control 0.254	261/90/4		
Male	MetS 0.327	124/64/8	1.78 (1.23-2.59)	0.002
	Control 0.224	170/50/3		
Female	MetS 0.338	100/52/2	1.17 (0.76-1.82)	0.472
	Control 0.303	91/40/1		

P value <0.05 considered as significant

MetS: Metabolic syndrome

MAF: Minor allele frequency

CI: Confidence interval

SNP: Single nucleotide polymorphisms.

**Table-4:** Clinical and laboratory data in patients with MetS and control subjects according to the APOA5 rs662799 genotypes.

Variables	Cases			Control		
	Non Carriers TT=224	Carriers (TC+CC=126)	P Value	Non Carrier TT=261	Carriers (TC+CC=94)	P value
Age (Years)	42.72±6.12	40.98±6.45	0.01	41.25±8.16	40.78±7.38	0.62
SBP (mm Hg)	129.95±14.13	130.56±14.49	0.81	115.88±8.69	115.71±9.64	0.88
DBP (mm Hg)	88.92±11.29	88.65±10.77	0.61	78.09±9.15	78.46±7.23	0.72
Waist(Inches)	39.21±2.19	38.96±2.11	0.34	32.47±1.59	32.44±1.38	0.85
Weight (Kg)	75.26±10.21	74.60±9.38	0.45	66.40±5.99	66.21±6.41	0.80
TC (mg/dl)	198.70±43.35	203.40±51.60	0.34	183.14±41.77	187.32±35.56	0.85
TG (mg/dl)	208.53±71.79	240.72±138.28	0.00	120.04±22.38	126.61±19.31	0.01
HDL (mg/dl)	38.56±8.14	36.32±10.63	0.02	44.97±6.54	45.45±5.30	0.51
LDL (mg/dl)	118.19±42.32	118.47±44.84	0.67	114.01±40.30	116.55±34.82	0.60
FBG (mg/dl)	144.79±73.34	150.62±83.35	0.59	92.50±10.73	91.36±11.53	0.38
BMI	28.23±4.81	27.64±4.61	0.29	23.740±2.72	23.73±2.11	0.88

SBP- Systolic Blood Pressure, DBP- Diastolic Blood Pressure, HDL-High Density Lipoprotein, LDL-Low Density Lipoprotein.

FBG- Fasting blood glucose, Chol-Cholesterol, TG-Triglyceride. TC: Total cholesterol.APOA5: Apolipoprotein A5 gene.

Values are given as mean ± SD.

P value <0.05 considered as significant.

There were significantly higher levels of systolic blood pressure (SBP), diastolic blood pressure (DBP), body weight, waist circumference (WC), BMI, total cholesterol (TC), TG, low density lipoprotein (LDL) and FBG among cases than controls, while there were low levels of HDL in cases than controls ( $p \leq 0.05$  each) (Table-1).

Genotype distribution followed Hardy-Weinberg equilibrium ( $p=0.1729$ ) in both cases and controls. Overall

genotypic distribution of rs662799 in the study cohort as well as MAF was noted (Table-2). The overall MAF (C) was 0.331 (Table-3). Significant association was found of minor C allele with high TG levels ( $p=0.0001$ ) and low HDL levels ( $p=0.02$ ) (Table-4).

Risk allele C of rs662799 also had strong association with obesity ( $p=0.01$ ) and DLP ( $p=0.03$ ), while there was no significant association with HTN ( $p=0.66$ ) (Table-5).

**Table-5:** Association of rs662799 SNP with Risk Components of MetS.

Phenotype	Odds Ratio	P Value
Dyslipidaemia	1.49(1.02-2.01)	0.03
Hypertension	1.13(0.71-1.77)	0.66
Obesity	1.46(1.09-2.08)	0.01

P value <0.05 considered as significant

SNP: Single nucleotide polymorphism

MetS: Metabolic syndrome.

## Discussion

In the present study the association of rs662799 SNP of APOA5 gene with IDF-defined<sup>16</sup> MetS and their components in a Pakistani population were explored. APOA5 is considered a strong determinant of plasma TG levels, which is one of the components of MetS and is among several MetS and HTG susceptibility genes.<sup>20,14</sup> The APOA5 affects TG levels by reducing the secretion of LDL from liver and increasing the metabolism of VLDL. The rs662799 of APOA5 was found to be associated with MetS and its major components like DLP and obesity in our study.

The association of rs662799 of APOA5 with MetS through its minor C allele in our study is consistent with previous studies showing significant association of rs662799 with MetS in Hungarians,<sup>21</sup> Romanians,<sup>22</sup> Chinese,<sup>13</sup> and Koreans.<sup>7</sup> It was found that association of C allele of rs662799 of APOA5 with MetS, like in our study, was mainly due to the association of minor C allele of rs662799 of APOA5 with obesity and HTG which are major determinants of MetS in Hungarian and Romanian populations.<sup>21,22</sup>

However, no such association of the C allele was found in Germans, Austrians<sup>15</sup> and in Turkish populations.<sup>23</sup> Further studies in Korean and Japanese populations also indicated that subjects carrying rs662799 homozygous CC genotype were more susceptible to MetS compared to TT and TC genotype carriers.<sup>7,14</sup> As rs662799 is not always associated with HTG or MetS, it is important to confirm this association in different populations, especially because the minor allele can be prevalent in certain ethnic populations and may confer considerable cardio-metabolic risk.

As shown in our study, rs662799 of APOA5 was associated with DLP and obesity, the major risk factors of MetS. DLP is characterised by presence of high TG and low HDL levels. This study showed significant prevalence of minor C allele of rs662799 of APOA5 among subjects having high TG and low HDL levels in Pakistani population. Our results are consistent with studies in Pakistani and

Japanese populations showing higher levels of TG in subjects carrying minor C allele of rs662799 of APOA5 gene.<sup>24,14</sup> Studies in Taiwanese and Hong Kong Chinese also confirmed that association of rs662799 with MetS becomes insignificant when adjusted for TG.<sup>25</sup> These results showed that rs662799 might be indirectly playing a role towards MetS susceptibility through its association with HTG. Positive association of rs662799 of APOA5 with obese subjects in our study is consistent with a study in the Romanian population where 70% of C allele-carrying MetS subjects were obese with high BMI compared to subjects lacking the C allele.<sup>22</sup>

The importance of a polymorphic genetic risk factor depends upon the MAF of risk allele in any ethnic population. It is noteworthy that the prevalence of the minor C allele of rs662799 of APOA5 is more prevalent in Asian populations. The MAF (C allele) in our studied Pakistani population was 0.331, which was similar to the MAF reported in other Asian ethnic groups,<sup>7,13,14</sup> but differed from Europeans, Americans,<sup>9</sup> Caucasians<sup>15</sup> and Turkish.<sup>23</sup> The C allele of rs662799 is more prevalent in East Asians compared to Caucasians, thus contributing to a higher risk of developing MetS.<sup>26,7</sup>

Gender-specific analysis showed that the association of APOA5 with MetS was more significant in male compared to female subjects in our Pakistani sample population, which was also the case in Chinese male subjects.<sup>26</sup>

## Conclusion

SNP rs662799 in the APOA5 gene was found to be significantly associated with DLP, obesity and MetS in Pakistan samples. The identification of carriers of the minor C allele of the APOA5 polymorphism rs662799 may prove helpful in predicting MetS susceptibility, especially in males who were found to be at a higher risk of developing MetS.

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**Competing Interests:** None.

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