Association of Angiopoietin-Like Protein-8 Gene Variant (Rs2278426 (C/T)) in a Cohort of Egyptian Patients with Metabolic Syndrome: A Case-Control Study

ABSTRACT

Objective: This study was conducted to reveal the association of ANGPTL8 gene variant (Rs2278426 (C/T)) with metabolic syndrome in a cohort of Egyptian patients.

Materials and methods: This study is a case control study that included 150 patients with metabolic syndrome and 150 healthy control subjects. All subjects were submitted to history taking and thorough physical examination and laboratory analysis. Genomic DNA was extracted and ANGPTL8 gene SNP (rs2278426) was detected by 5' nuclease assay. The tested genotypes included homozygous genotypes for C allele (CC), homozygous genotypes for T allele (TT) and heterozygous genotypes (CT).

Results: A total of 300 subjects were included in the study; group 1 included 150 patients with metabolic syndrome. 21.3% were males and 78.7% were females, and group 2 had 150 healthy subjects. 17.3% were males, and 82.7% were females. In the current study, the metabolic syndrome group showed dysregulation of lipids and fasting plasma glucose (FPG) with a statistically significant increase in body anthropometric measures. There was no statistically significant difference in the distribution of the heterozygous genotype (C/T) relative to the wild-type genotype (C/C) in each of the two tested groups (p = 0.287 and 0.245 in the metabolic syndrome and control groups, respectively).

Conclusions: There was no statistically significant difference in the genotype distribution of ANGPTL8 gene variant (Rs2278426) genotypes between the metabolic syndrome and control groups. The homozygous recessive genotype for T allele (TT) was not detected in both tested groups. (Clin Diabetol 2023; 12; 4: 215–222)

Keywords: metabolic syndrome, ANGPTL8, rs2278426, genotyping, polymerase chain reaction (PCR)

Introduction

Metabolic syndrome (MetS) is considered a major risk factor for cardiovascular diseases and stroke in both developing and developed countries. Central obesity and type 2 diabetes (T2D) are considered the major endophenotypes of MetS, which are inheritable and genetically based [1].

In Egypt, 22% of men and 48% of women were obese, according to a 2010 World Health Organization (WHO) estimate. The Middle East and North Africa Region is considered to have the highest prevalence of diabetes at 10.9% [2].
Angiopoietin-like proteins (ANGPTLs) are proteins that are primarily expressed in the liver and adipose tissue and circulate in human plasma. Expression of ANGPTL8 was found to be reduced by fasting and increased by re-feeding in both mice and humans [3]. ANGPTL8 has been associated with two functionally important processes in the development of T2D, insulin resistance and lipid metabolism, and has also been reported to regulate the replication of β-cells in response to insulin resistance [4, 5].

Overexpression of ANGPTL8 leads to increased plasma levels of triacylglycerol (TAG) and lipoproteins via inhibition of LPL activity [6, 7]. Therefore, ANGPTL8 has been suggested as a potential therapeutic target for dyslipidemia, and inhibition of ANGPTL8 has been highlighted as a novel therapeutic strategy for reducing plasma lipoprotein levels [8].

C > T (rs2278426) is a commonly studied variant in ANGPTL8 gene located on chromosome 19, resulting from the substitution of C for T at c194, that affects levels of the activated form of ANGPTL3 with a similar effect to complete ANGPTL3 deficiency [9].

Due to this suggested association, the current study was conducted to reveal the association of ANGPTL8 gene variant (Rs2278426 (C/T)) with metabolic syndrome in a cohort of Egyptian patients.

**Materials and methods**

**Study design**

This is a case-control study that was conducted at the Diabetes and Metabolism Clinic at the Internal Medicine Department, Alexandria University Hospitals, Alexandria, Egypt, for a duration of 1 year between January 2022 and December 2022.

**Study population**

This study included a total of 300 subjects who were divided into two groups: Group 1 (the study group), which included 150 patients with metabolic syndrome, and Group 2 (the control group), which included 150 apparently healthy persons.

**Ethical approval**

The study was conducted in accordance with Helsinki Standards as revised in 2013 [10]. The study was conducted after obtaining approval from the local ethics committee, Faculty of Medicine, Alexandria University, and after obtaining a written or oral informed consent from the included cases.

The participants were invited from the outpatient clinics of the diabetes and metabolism unit at the Internal Medicine Department, Alexandria University Hospitals, Alexandria, Egypt, for a duration of 1 year between January 2022 and December 2022.

After explanation of the study purpose an informed written consent was obtained from all the participants. The subjects were informed about their right to withdraw at any time from the study.

**Inclusion criteria**

We included the patients from both genders aged more than 18 years who were diagnosed to have metabolic syndrome. According to the IDF definition, metabolic syndrome is diagnosed if anyone has central obesity plus two or more of the following four criteria [11]: Triglyceride concentration > 150 mg/dL, high density lipoprotein cholesterol (HDL-C) < 40mg/dL in men and < 50 mg/dL in women, or receiving lipid lowering drugs, systolic blood pressure > 130 mmHg or diastolic blood pressure > 85 mmHg or receiving treatment for hypertension and fasting plasma glucose > 100 mg/dL or previously diagnosed type 2 diabetes.

**Exclusion criteria**

The study excluded cases with the following criteria: age < 18 years, patients with type 1 diabetes, patients with acute infection or illness, malignancy, liver cell failure, kidney failure, heart failure, unstable medical condition during the previous 30 days, patients with associated active inflammatory conditions and gastrointestinal diseases, psychological disease and patients who refused to participate in the study.

The cases were subjected to the following: history taking, a thorough physical examination, and a complete clinical history and clinical examination. Anthropometric measures of all the subjects in the study were obtained, including weight, height, body mass index (BMI), and waist circumference.

Body mass index is defined as the weight in kilograms divided by the square of the height in meters (kg/m²). BMI values between 18.5 and 24.99 kg/m² are considered normal; individuals with BMI values from 25 to 29.9 kg/m² are considered overweight; and those with values greater than 30 kg/m² are considered obese [12].

Waist circumference (a measurement taken around the abdomen at the level of the umbilicus) The NIH was the first to use the threshold values for waist circumference (≥ 88 cm in women and ≥ 102 cm in men) as suggested by Michael Lean and colleagues in combination with a classification of overall obesity as assessed by BMI [13].

**Laboratory data**

Lipid profile [triglyceride (TG) (normal: less than 150 milligrams per deciliter (mg/dL), total cholesterol (TC) (normal: less than 200 mg/dL), high density lipo-
protein cholesterol (HDL-C) (60 mg/dL or above), and low-density lipoprotein cholesterol (LDL-C) (the normal range is 50 to 100 mg/dL) and diabetic profile [fasting plasma glucose (FPG) (a fasting plasma glucose level of 99 mg/dL or lower is normal), glycated hemoglobin (HbA1c) (normal HbA1c level is ≤ 5.6, pre-diabetes 5.7–6.4 and diabetes ≥ 6.5 or below), and fasting serum insulin (normal fasting insulin levels range between 5 and 15 µU/mL)].

Assessment of the insulin resistance (IR) index by using Homeostatic Model Assessment of insulin resistance (HOMA-IR) equation: HOMA-IR = fasting insulin (µU/mL) × fasting glucose (mg/dL) /405 [14].

Blood sampling

All blood samples were divided into two aliquots: the first part was collected in a vacutainer tube containing Na2-EDTA for the assay of HbA1c; second part was collected in a plain vacutainer tube and centrifuged (3000 rpm) for serum preparation. Serum was used to measure total cholesterol, triglycerides, LDL cholesterol, HDL cholesterol, and glucose. Serum was used to measure total cholesterol, triglycerides, LDL and HDL cholesterol, glucose. Low-density lipoprotein cholesterol was calculated by using Friedewald formula:

\[ \text{TC} - (\text{HDL-C}) - (\text{TG}/5) \] [14].

Fasting serum insulin (µU/mL) was measured by ELISA from the serum sample. Assessment of insulin resistance (IR) index was made using Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) equation:

\[ \text{HOMA-IR} = \text{fasting insulin (µU/mL)} \times \text{fasting glucose (mg/dL)}/405. \]

Genetic analysis

**ANGPTL8** gene SNP (rs2278426) detection by allelic discrimination using a fluorogenic 5’ nuclelease assay

a) DNA extraction:

Genomic DNA was extracted from an EDTA blood sample using the QiAamp DNA Mini Kit according to the manufacturer’s instructions (Qiagen, Germany). The extracted DNA concentration and purity were assessed using a NanoDrop 2000/2000c spectrophotometer (Thermo Scientific, USA). Ratios of 260/280 and 260/230 = 1.7–2 indicated highly pure DNA.

b) 5’ nuclelease assay:

**ANGPTL8** gene SNP (rs2278426) was detected by 5’ nuclease assay using TaqMan® Genotyping Master Mix, TaqMan® SNP Genotyping Assay 40x (Assay ID: C_15965472_10) (Thermo Fisher Scientific, USA), and Rotor-gene Q real time polymerase chain reaction (PCR) system (Qiagen, Germany).

**Principle**

Real-time PCR utilizes the 5’ to 3’ exonuclease activity of Taq polymerase as the enzyme cleaves the 5’ terminal nucleotide of double stranded DNA. A fluorescent oligonucleotide TaqMan probe is designed to anneal between the two amplification primers in the PCR reaction. This probe is labeled with two fluorescent dyes, a reporter dye at the 5’ end and a quencher dye at the 3’ end. The quencher exerts a quenching effect on the fluorescence of the reporter dye as long as the probe is intact and the two dyes are in close proximity.

During extension, Taq polymerase partially displaces the hybridized probe and then cleaves it at the 5’ end. The cleavage of the probe between the reporter and the quencher physically separates the two dyes, resulting in increased reporter fluorescence, which indicates that the probe-specific target has been amplified.

For allelic discrimination, two probes with different reporters were used: one gave a green color called “FAM” and the other gave a yellow color called “VIC”. An increase in the fluorescence signals of a particular dye indicates homozygosity for the allele whose probe was labeled by that dye; an increase in both signals indicates heterozygosity.

**Statistical analysis**

The data collected were coded, processed, and analyzed with SPSS version 26 for Windows® (Statistical Package for Social Sciences) (IBM, SPSS Inc., Chicago, IL, USA). Qualitative data in the form of numbers (frequency) and percent was presented. The Chi-Square test (or Fisher’s exact test) made the comparison between groups. The Kolmogorov-Smirnov test tested quantitative data for normality. Parametric data were shown as median ± SD, while non-parametric data were expressed as median (interquartile range).

To compare two groups with normally distributed quantitative variables, an independent sample (Student’s) t-test was used, and a Mann-Whitney U-test was used if the data were abnormally distributed. The Hardy-Weinberg Equilibrium equation was used to assess the genotype frequency distribution in each of the two tested groups. For all tests, P values < 0.05 are considered significant.

The sample size was estimated based on the Epi Info V 7.0 program with a power of 80 and a level of significance of 95%, considering the percent of exposed to the outcome of 16% and the percent of unexposed to the outcome of 7.4%. The program revealed...
a minimum sample size of 246 individuals, 123 for each group. However, this study will be conducted on a sample size of 300 subjects for the purpose of improving the validity of the research results.

**Results**

Table 1 shows that there was no statistically significant difference between the two groups regarding the age and sex. The BMI and waist circumference were statistically significantly higher in the metabolic syndrome group as compared to the control. In the metabolic syndrome group, the prevalence of diabetes was 29.3% and the prevalence of hypertension was 66.7%.

The mean systolic blood pressure and the mean diastolic blood pressure were statistically significantly higher in the metabolic syndrome group as compared to the control. The mean high density lipoprotein level was statistically significantly lower in the metabolic syndrome group as compared to the control.

Regarding the distribution of the genotype of the tested gene in the two groups; there were 126 cases (84%) with wild type form and 24 (16%) with heterotype form in the metabolic syndrome group, while in the control group, wild type was detected in 82.7% and heterotype was detected in 17.3%, with no statistically significant difference between the two groups (p = 0.757).

Also, by using Hardy-Weinberg Equilibrium for genotype analysis, there was no statistically significant difference in the distribution of the heterozygous genotype (C/T) relative to the wild type genotype (C/C) in each of the two tested groups (p = 0.287 and 0.245 in the metabolic syndrome and control groups, respectively).
Table 2 shows that there was no statistically significant difference between the wild type and heterotype genotypes in all the tested clinical and laboratory parameters except for HDL-C.

The mean level of HDL-C in the hetero (C/T) genotype was 46.13 ± 5.45 mg/dL, which was statistically significantly higher compared to the wild type genotype (43.17 ± 5.33 mg/dL) (p = 0.014).

On the other hand, in Finland, the prevalence of MetS was higher in males compared with females in a study conducted on subjects between 45 and 64 years old, as the occurrence of MetS was 52.6% among males and 45.3% among females [17]. The variations could be explained by variations in population characteristics.

In the current study, the prevalence of hypertension in the metabolic syndrome group was 66.7%, and 42% only received hypertension treatment. The mean SBP and DBP were statistically significantly higher in the cases with metabolic syndrome compared to the controls. This agreed with Abd Elaziz et al. [2] who included 220 middle-aged and senior Egyptians who were voluntarily screened in an Egyptian private hospital. The authors observed that ~ 76.6% of hypertensive patients had MetS.

In the current study, the prevalence of T2D in the metabolic syndrome group was 29.3%. Also, the fasting insulin level, fasting plasma glucose, HbA1c, and HOMA-IR were statistically significantly higher in the

<table>
<thead>
<tr>
<th>Wild (n = 126)</th>
<th>Hetero (C/T) (n = 24)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>100 (79.4%)</td>
<td>18 (75.0%)</td>
</tr>
<tr>
<td>Age [years] (mean ± SD)</td>
<td>42.9 ± 10.8</td>
<td>39.4 ± 8.9</td>
</tr>
<tr>
<td>BMI [kg/m²] (mean ± SD)</td>
<td>39.8 ± 6.2</td>
<td>38.8 ± 6.6</td>
</tr>
<tr>
<td>Waist circumference [cm] (mean ± SD)</td>
<td>115.5 ± 9.3</td>
<td>114.4 ± 9.5</td>
</tr>
<tr>
<td>SBP [mmHg] (mean ± SD)</td>
<td>130.1 ± 18.9</td>
<td>125.8 ± 11.8</td>
</tr>
<tr>
<td>DBP [mmHg] (mean ± SD)</td>
<td>86.8 ± 12.0</td>
<td>85.6 ± 10.1</td>
</tr>
<tr>
<td>Fasting insulin (mean ± SD)</td>
<td>15.4 ± 4.3</td>
<td>14.7 ± 4.4</td>
</tr>
<tr>
<td>FPG [median (IQR)]</td>
<td>107.0 (71.0–361.0)</td>
<td>100.0 (63.0–172.0)</td>
</tr>
<tr>
<td>HbA1c [%] (mean ± SD)</td>
<td>6.3 ± 1.3</td>
<td>6.2 ± 1.1</td>
</tr>
<tr>
<td>HOMA-IR [median (IQR)]</td>
<td>2.1 (0.6–6.6)</td>
<td>1.8 (1.0–3.5)</td>
</tr>
<tr>
<td>TGs [median (IQR)]</td>
<td>139.0 (53.0–298.0)</td>
<td>145.5 (71.0–301.0)</td>
</tr>
<tr>
<td>TC (mean ± SD)</td>
<td>207.6 ± 35.5</td>
<td>204.4 ± 32.16</td>
</tr>
<tr>
<td>LDL-C (mean ± SD)</td>
<td>135.1 ± 34.3</td>
<td>129.8 ± 32.8</td>
</tr>
<tr>
<td>HDL-C (mean ± SD)</td>
<td>43.2 ± 5.3</td>
<td>46.1 ± 5.4</td>
</tr>
</tbody>
</table>

*Statistically significant at p ≤ 0.05

BMI — body mass index; DBP — diastolic blood pressure; FPG — fasting plasma glucose; HbA1c — glycated hemoglobin; HDL-C — high-density lipoprotein cholesterol; HOMA-IR — Homeostatic Model Assessment for Insulin Resistance, , IQR — interquartile range; LDL-C — low-density lipoprotein cholesterol; SBP — systolic blood pressure; SD — standard deviation; TC — total cholesterol; TG — triglycerides
metabolic syndrome group. This agreed with Abd Elaziz et al., who showed that the prevalence of MetS among people with diabetes in their study was 85.6%. This was also similar to a study done on Pakistani patients with T2D, where 85.5% of the people with diabetes had MetS [21].

In the current study, the mean TGs level, TC level, and LDL-C were statistically significantly higher in the cases with metabolic syndrome, while the HDL-C was statistically significantly lower in the cases group compared to the control. This came in accordance with Refaat et al. [22], who included 100 Egyptian patients selected by random sampling technique (males and females), diagnosed as having metabolic syndrome, and fifty apparently healthy volunteers recruited from paramedical personnel, served as controls. They showed higher levels of TGs, LDL, and cholesterol in the cases with metabolic syndrome and a lower level of HDL compared to the controls.

The circulating level of ANGPTL8 was significantly higher in subjects with MetS (metabolic syndrome) as well as subjects with an increasing number of MetS components such as insulin resistance and central obesity. ANGPTL8 also showed a significant association with hsCRP, BMI, TG, LDL, HOMA-IR, and FPG and was associated with a 2.4-fold increase in having MetS [23].

However, there is a shortage of studies concerning the association between gene polymorphisms and metabolic syndrome.

In the current study, regarding the distribution of the genotype of ANGPTL8 gene variant (Rs2278426) in the two groups; there were 126 cases (84%) with wild type form and 24 (16%) with heterotype form in the metabolic syndrome group, while in the control group, wild type was detected in 82.7% and heterotype was detected in 17.3%, with no statistically significant difference between the two groups (p = 0.757). The current results were similar to those of Alenad et al., who included a total of 905 unrelated Saudi adults (580 healthy controls and 325 MetS). The genotype and allele frequency distribution of rs737337 (T/C) and rs2278426 (C/T) polymorphisms in ANGPTL8 gene were studied. The results showed that the genotype/allele frequency distribution of rs2278426(C/T) was comparable in both study groups (p > 0.05) [24]. The current results disagreed with El-Lebedy, who included 272 subjects classified into 68 patients with T2DM, 68 patients with T2D + CVD, and 136 control subjects. The results showed that ANGPTL8 c194C>T (rs2278426), the frequency of the variant allele (T), was significantly higher in T2DM patients than in control subjects (16% vs. 7.4%, p = 0.001) and was associated with a 2.33-times increased risk of T2DM (95% CI 1.33–4.08) [25].

This also contradicts those of Guo et al., who included a total of 217 T2D patients and 201 healthy control subjects with normal glucose tolerance (NGT) in their study. The authors showed that the frequency of the CT + TT genotype in the T2D group (45.6%) was significantly higher than in the NGT group (34.8%). Relative-risk analysis showed that the CT + TT genotype was a risk factor for T2DM (OR 1.57, 95% CI 1.05–2.32) [26].

Also, the current study disagreed with those of Huo et al., who included 1,460 participants in this case-control study: healthy controls, n = 524; pre-DM, n = 460; and T2D, n = 460. The results showed that in T2D patients, the frequency of the CT (48%) and TT (15%) genotypes was higher than that in the control group (CT: 40.1% and TT: 6.8%). After adjusting for age, sex, and BMI, the ORs were 1.384 for the CT genotype (95% CI = 1.013–1.890) and 2.530 for the TT genotype (95% CI = 1.476–4.334) [27].

Other studies reached the same conclusion opposite to ours. A study found that, in the Iranian population, the frequency of the CT gene at this locus was significantly higher in patients with T2D than in the control group, suggesting that this genotype increased the risk of T2D [28]. Additionally, a similar study of the rs2278426 polymorphism in the Japanese population found that a higher proportion of patients with T2D and impaired glucose tolerance carried the CT or TT genotypes compared with the CC genotype [29].

The variations may be mostly due to ethnic differences, different grouping indicators, and variations in MAF (minor allele frequency).

In the current study, there was no statistically significant difference between the wild type and heterotype genotypes in all the tested clinical and laboratory parameters except for HDL-C. The mean level of HDL-C in the hetero (C/T) genotype was 46.13 ± 5.45 mg/dL, which was statistically significantly higher compared to the wild type genotype (43.17 ± 5.33 mg/dL) (p = 0.014).

The results of the association between the genotypes and the diabetic and lipid profiles were contradictory.

Ethnic variation has been associated with variable minor allele frequency (MAF) rates for this variant with respect to LDL-C and HDL-C plasma levels. In the Dallas Heart Study (DHS), a multiethnic population-based study of Dallas County, Hispanics had the highest MAF of 26%, followed by African-Americans (18%), and the least was reported in European-Americans (5%). ANGPTL8 rs2278426 was associated with lower LDL-C and HDL-C in African-Americans and Hispanics but
not in European-Americans, and was not associated with fasting plasma glucose or homeostatic model assessment-insulin resistance in any ethnic group [9]. However, in a genome-wide association study that predominantly included individuals of European ancestry, this variant was associated with both HDL-C and LDL-C [30]. Meanwhile, in a study on non-diabetic Arabs, individuals who carried this variant had higher FPG, but did not show significant differences in their LDL and HDL levels compared to the wild type carriers [23].

In addition, Hanson et al. revealed that the T allele of the rs2278426 (C/T) variant is associated with decreased levels of total cholesterol and HDL-C in Pima Indians and Mexican Americans [31]. Furthermore, Ghasemi et al. demonstrated that the CT heterozygote genotype of the rs2278426 (C/T) polymorphism had higher levels of FPG, HbA1c, insulin, and insulin resistance compared to the CC and TT genotypes. In other words, CT genotype is associated with the risk factors of T2D. On the other hand, there was no significant relationship between this variant and HDL-C or LDL-C [28].

There is a disparity between reported results about ANGPTL8 rs2278426 (C/T) polymorphism and biochemical factors, which is mainly due to the differences in the races of the studied populations. In this regard, Guo et al. showed that differences in race can affect the allelic and genotypic frequency of ANGPTL8 rs2278426 (C/T) and also their relationship with other biochemical parameters [32].

Regarding the significant association between the heterotype genotype and higher HDL-C levels, previous studies have shown that ANGPTL3 inhibits lipoprotein lipase (LPL) activity [33, 34]. In addition, another study showed that inactivation of ANGPTL3 causes a significant reduction in HDL-C levels [35]. Therefore, it can be concluded that ANGPTL3 activity is necessary for HDL-C biogenesis. Quagliarini et al. demonstrated that ANGPTL3 activity required its interaction with ANGPTL8. Therefore, it is possible that any factor interfering with this interaction can decrease HDL-C levels [9].

This study had the limitation that it was conducted in a single center, “at the Diabetes and Metabolism Clinic at the Internal Medicine Department, Alexandria University Hospitals”, and therefore, generalization is limited.

Conclusions

Metabolic syndrome is a common health problem that affects Egyptian society. There was no statistically significant difference in the genotype distribution of ANGPTL8 gene variant (Rs2278426) genotypes between the metabolic syndrome and control groups. The homozygous recessive genotype for the T allele (TT) was not detected in both tested groups.

Funding

This study is self-funded.

Conflict of interest

None declared.

REFERENCES