**ORIGINAL ARTICLE, MEDICINE**

**Chemerin rs17173608 Gene Polymorphism is not Associated with Type 2 Diabetes Mellitus: a Cross-sectional Study**

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**Received:** 13 Jan 2018
**Accepted:** 22 May 2018
**Published online:** 08 Aug 2018
**Published:** 31 Mar 2019

**Key words:** chemerin, polymorphism, diabetes mellitus

**Citation:** Olt S, Öznas O, Bağış H, Turanlı ET. Chemerin rs17173608 gene polymorphism is not associated with type 2 diabetes mellitus: a cross-sectional study. Folia Med (Plovdiv) 2019;61(1): doi: 10.2478/folmed-2018-0045

**Background:** Previous studies have shown that chemerin has important roles in the development of obesity, insulin resistance, metabolic syndrome, polycystic ovary syndrome (PCOS) and T2DM. The main goal of our study was to investigate the role of Chemerin rs17173608 gene polymorphism in T2DM (type 2 diabetes mellitus).

**Materials and methods:** 100 patients with T2DM and 50 healthy volunteers were included in the present study. DNA isolation from blood samples was performed with K1820-02 DNA Mini Kit. Chemerin gene polymorphism was detected by Tetra-Amplification Refractory mutation system polymerase chain reaction (T-ARMS-PCR). At the end of T-ARMS-PCR, samples were run using gel electrophoresis. Some samples were validated by sequence analysis.

**Results:** In the genotype analysis, 18.0% of patients had TT genotype and 81.0% of TG genotype was detected. GG genotype was not detected in any patient. Genotype of 1 patient was unidentified. Genotype distribution of healthy control group was 12.0% TT genotype and 88.0% TG genotype. Similar to the T2DM group, the GG genotype was not detected in the control group. There was no statistically significant difference between T2DM group and healthy control group for TG and TT genotypes.

**Conclusion:** To our knowledge, chemerin rs17173608 gene polymorphism has been investigated in T2DM for the first time herein. In the present study, the TT genotype ratios were higher in the T2DM subjects than in healthy subjects. G allele frequency in the T2DM group was lower than that in the control group. However, there was no statistically significant difference between the groups.

**BACKGROUND**

T2DM is a chronic disease characterized by disturbance of carbohydrate, fat and protein metabolism and abnormal blood glucose levels. The quality of life and survival of patients are directly affected by early and late complications. As in the past 20 years, there is a serious increase in DM prevalence in our country as well.¹ The proportion of T2DM is particularly high in urban and developed societies, where obesity, sedentary life and industrialization are prevalent. In conclusion of the imbalance in energy metabolism, the proportion of fat mass in the body to fat-free body mass is an important risk factor for obesity, and the majority of diabetic patients are obese. The rapid socioeconomic development, the increase in average life expectancy and the increase in ready food consumption have reinforced the obesity ground. It has been estimated that 150 million people in the world are suffering from T2DM, this number predicted to reach 380 million in the next 10 years.² Early diagnosis and prevention studies have become important for DM, which has become a pandemic problem in the light of these data.

Until recently, it has been shown that fat tissue, which is thought to have only energy storage and protective barrier functions, is an active endocrine organ that secretes hormones in conclusion of scientific investigations. Adipose tissue plays a role in many metabolic events. The number of adipokines nowadays is more than 20 and this number is increasing every day.³ Through these mediators, they play a role in many physiological processes of the body such as nutrition, appetite, energy balance,
insulin and glucose metabolism, lipid metabolism, regulation of blood pressure, vascular remodeling, coagulation and inflammation. Adipokines regulate appetite and energy consumption affecting insulin sensitivity, oxidative capacity and lipid uptake in the periphery. Some of these peptides are TNF-alpha, IL-6, resistin, leptin, adiponectin, vaspin, chemerin, visfatin and omentin. Today, scientists are getting increasingly interested in these molecules. Adipokines are primarily associated with obesity and pathological processes related to obesity.

One of the newest member of the adipokines family discovered in recent years is chemerin. This adipokine secreted from adipose tissue is effective on cells expressing receptor known as chemokine-like receptor 1 (CMKLR1) or ChemR23, and there are two other known receptors. Chemerin was first discovered as a chemotactic peptide in macrophages expressing ChemR23 in inflammatory processes and in dendritic cells. Chemerin, which appears to have pleiotropic functions, has been classified as an adipokine because it allows differentiation of adipokines and is involved in glucose uptake. Chemerin is activated by serine proteases during inflammation and coagulation and stimulates the chemotaxis of macrophages and dendritic cells to the inflamed area. It has also been shown that chemerin has a direct pro- and anti-inflammatory effect. Studies have shown that chemerin has important roles in the development of obesity, insulin resistance, metabolic syndrome, polycystic ovary syndrome (PCOS) and T2DM.

**AIM**
The aim of this study was to investigate the relationship between the levels of chemerin gene polymorphism and chemerin protein expression and T2DM.

**MATERIALS AND METHODS**

**SUBJECTS**
One hundred patients with T2DM and 50 healthy volunteers were included in the study between July 2016 and May 2017.

**PATIENTS’ GENERAL CHARACTERISTICS DESCRIPTION**
The mean age of the patient group was 55±9 years. The mean diabetes duration of diabetic patients was 8.3±5.5 years. Body mass index averaged 30.4±6.3 kg/m². The mean HbA1C was 8.7±2.3 %. 71% of the patients were female, 29% were male. The mean age of the healthy group was 46±14. 70% of the control group were female, 30% were male.

**INCLUSION CRITERIA**
- Individuals over 18 years of age.
- DM group will consist of the T2DM diagnosed patients. DM diagnosis was made with double-starvation blood glucose levels ≥126.

**EXCLUSION CRITERIA**
- Presence of a chronic illness in the control group.
- Individuals under 18 years of age.

**MATERIALS AND METHODS**
DNA isolation from blood samples was performed with the K1820-02 DNA Mini Kit at Adiyaman University Medical Faculty Medical Genetics Laboratory. Chemerin gene polymorphism was detected by Tetra-Amplification Refractory mutation system polymerase chain reaction (T-ARMS-PCR) in Molecular Biology and Genetics Department of Istanbul Technical University. At the end of T-ARMS-PCR, samples were run using gel electrophoresis. Some samples were validated by sequence analysis.

The ARMS-PCR method was used to determine the genotypic differences of the chemerin broad rs17173608 polymorphism. At the end of the ARMS-PCR, the samples were run on a 2% agarose gel at 110 V for 35 min using gel electrophoresis. The genotypes of patient and healthy controls were determined by looking at the gel images. The image of the gel prepared by Hashemi M et al. was used as a reference for genotyping (Fig. 1).

**STATISTICAL ANALYSIS**
Statistical analyses were performed with the programmed Statistical Package for Social Sciences (SPSS) for Windows version 15.0. When the data were evaluated, the continuous variables were

![Figure 1. T-ARMS-PCR for detection of chemerin rs17173608. M=100 bp DNA markers.](image)
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expressed as mean ± standard deviation and the frequency was given as the number (%). Chi square test was used for statistical analysis. P<0.05 was considered statistically significant.

**ETHICAL STATEMENT**

Ethics Committee approval was obtained from the Ethics Committee of the Scientific Researches of Adiyaman University Faculty of Medicine on 01.20.2016 and numbered 1/17. The study was conducted in a manner consistent with the Declaration of Helsinki.

**RESULTS**

**GENETIC ANALYSIS**

In the genotype analysis, 18.0% of diabetic patients had TT genotype and 81.0% of TG genotype was detected. Genotype of 1 patient was unidentified. However, the GG genotype was not detected in any of the patients. In the control group genotype distribution, 12.0% TT genotype and 88.0% TG genotype were detected. Similar to the patient group, the GG genotype was not detected in the control group. When the patients and control groups were compared statistically, there was no statistically significant difference in genotype (P = 0.249). On the other hand, the T allele frequency was calculated as 59.1% and the G allele frequency as 40.9% in the patient group. In the control group, T and G allele frequencies were calculated as 56.0% and 44.0%, respectively. There was no statistically significant difference between groups in terms of T and G alleles. Detailed information on genotype analysis is shown in Table 1.

**SEQUENCE CONFIRMATION OF NON-ANALYZABLE SAMPLES IN GEL ELECTROPHORESIS**

Sequence results were analyzed by the Genius program after the PCR was established from 2 patients (Patient-9 and Patient-10) and 2 healthy controls (Healthy Control-26 and Healthy Control-27) to send Sanger sequence.

**FOR PATIENT-9**

G / G was homozygous based on the reference article. Sanger sequence results showed that the base was T and the result was actually T / T homozygote (Fig. 2).

**FOR PATIENT-10**

Based on the reference article, T / G was evaluated as a heterozygote. Sanger sequence results were confirmed as T / G heterozygote for that base. Only forward reading was performed, and baseline shift occurred, and a chromosomal T / G heterozygous base was observed on the chromatogram (Fig. 3).

**FOR HEALTHY CONTROL-26**

G / G was homozygous based on the reference article. Sanger sequence results showed that the base was T and the result was actually T / T homozygote (Fig. 4).

| Chemerin rs17173608 polymorphism | Diabetic group n (%) | Healthy group n (%) | p value |
|----------------------------------|----------------------|---------------------|--------|
| **Codominant**                  |                      |                     |        |
| TT                              | 18 (18.0)            | 6 (12.0)            | 0.24   |
| TG                              | 81 (81.0)            | 44 (88.0)           |        |
| GG                              | 0 (0.0)              | 0 (0.0)             |        |
| Unidentified                    | 1                    |                     |        |
| **Dominant**                    |                      |                     |        |
| TT                              | 18 (18.0)            | 6 (12.0)            | 0.24   |
| TG+GG                           | 81 (81.0)            | 44 (88.0)           |        |
| **Recessive**                   |                      |                     |        |
| TT+TG                           | 99 (99.0)            | 50 (100.0)          | 1.00   |
| GG                              | 0 (0.0)              | 0 (0.0)             |        |
| **Allele**                      |                      |                     |        |
| T                               | 117 (59.1)           | 56 (56.0)           | 0.54   |
| G                               | 81 (40.9)            | 44 (44.0)           |        |
Figure 2. P-9 Sanger sequence analysis result.

Figure 3. P-10 Sanger sequence analysis result.

Figure 4. HC-26 Sanger sequence analysis result.

Figure 5. HC-27 Sanger sequence analysis result.
Based on the reference article, J / L was evaluated as T / G heterozygote. But in the gel image the bands were not very clearly distinguishable. Sanger sequence results showed that the result is actually T / T homozygote (Fig. 5).

DISCUSSION

DM is one of the most common chronic disease that causes damage to many organs and systems. Individuals with genetic predisposition develop DM when exposed to a variety of environmental factors. As a basis for the treatment, fasting blood sugar should be brought to ideal levels. Many complications can develop in many tissues if the necessary interventions are not implemented. These can develop as macro-vascular and micro-vascular complications. Macro-vascular complications are: peripheral arterial disease, diabetic foot, stroke, cardiovascular diseases; micro-vascular complications are: neuropathy, retinopathy, nephropathy. Vascular complications can begin many years before diagnosis. The quality of life and social life of patients are negatively affected by the financial and moral burden of complications. Preventable complications are possible with early diagnosis and tight glycemic control. Various mechanisms and inflammatory processes are important in the development of complications and in the pathogenesis of the disease.9,10

In recent years, it has been observed that the adipokines family is increasingly concerned by scientists in insulin resistance, metabolic syndrome, hyperlipidemia, DM and cardiovascular diseases. Fat tissue is an endocrine organ that produces adipokines. Gimble et al.11 and Trayhurn et al.12 found that the amount of fat tissue was related to insulin resistance, metabolic syndrome, hyperlipidemia, cardiovascular diseases and T2DM as well as adipokines have been reported to play an important role in the pathogenesis of these diseases. Goralski et al. demonstrated that presumably they were secreted from adipose tissue and that they were particularly effective in direct or indirect insulin sensitivity and sensitivity to various pathways.13 Knowing adipokines and their functioning specifically for DM may help us prevent disease and diabetes related complications.

Chemerin is released from white fat tissue and thought to be responsible for adipogenesis. In addition, it has been found that it has roles in regulating inflammation and insulin sensitivity in fat tissue. In a study by Lago et al., it was emphasized that chemerin could be responsible for the development and chronic progression of some inflammatory diseases such as obesity and T2DM.14

Ouwens et al. found that chemerin positively correlated with insulin resistance.15 Chemerin can be used as a marker for insulin resistance in healthy individuals who are non-obese and non-metabolic disorders.

In a study conducted by Parolini et al.16 and Takashi et al.17, positive correlations were found between serum levels of chemerin expression in fat tissue and serum chemerin concentration. In studies conducted by Du et al.18 and Albanesive et al.19, positive correlation was found between serum chemerin levels and inflammatory cytokines, body mass index (BMI) and metabolic syndrome.

Dranse et al.20 reported that chemerin induces nuclear factor-dependent B-dependent pathways through expression to regulate adipose tissue remodeling and inflammation. Furthermore, a study conducted by Methanna et al. found higher fasting glucose, insulin and HOMA-IR levels in rs17173608 G allele carriers.21 In studies conducted by Weigert et al.22 and Tomalka et al.23, higher levels of chemerin were observed in many immuno- and inflammatory diseases (ulcerative colitis and Crohn’s, multiple sclerosis, psoriasis, rheumatoid arthritis). This suggests that chemerin is associated with systemic inflammation.

Genetic polymorphism is usually a sequence variation due to the change of single base pair. The credibility of genetic research studies and developments in genotyping have increased the interest of the researchers in this area. These methods can specifically indicate the level of genetic diversity and variations. Genetic variations may be related to chronic disease duration, behavior and complication characteristics.

Several gene expressions and gene polymorphisms have been studied to explain various mechanisms of DM.10,15,24 In a study conducted by Parolini et al., rats with T2DM showed more chemerin expression in visceral fat than control rats.16 As far as we know, our study is the first study that has investigated the association of chemerin rs17173608 gene polymorphism in T2DM.

Chemerin rs17173608 gene polymorphism was studied in different populations in previous studies. A significant association between the metabolic syndrome and the chemerin rs17173608 polymorphism has been shown in a study by Methanna et al.21 The G allele frequency was found to be
higher in the metabolic syndrome group. However, body mass index and waist circumference were observed more frequently in the G allele. Another study (Hashemi et al.) showed that the G allele had a risk for metabolic syndrome.8

In the present study, the TT genotype ratios were higher in the T2DM subjects than in healthy subjects. GG genotype were not observed in any subjects. G allele frequency in the T2DM group was lower than the control group. However, there was no statistically significant difference between the groups (P > 0.05).

CONCLUSION

Chemerin rs17173608 gene polymorphism has been investigated in T2DM patients in Adıyaman province of Southeastern Anatolia Region of Turkey for the first time. There was not any association between the chemerin rs17173608 gene polymorphism and T2DM. However, there were some limitations of our study. These were, small number of patients and lack of follow-up of the healthy controls as some of the healthy controls may be at high risk and may develop T2DM during the subsequent years. Because, some of the healthy controls may be at a higher risk and may develop T2DM during the following years. Thus, the future prospective studies will illuminate this point.

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