ORIGINAL ARTICLE / ARAŞTIRMA MAKALESİ

# Investigation of Gene Polymorphisms of *Vaspin, Visfatin* and *Chemerin* in Diabetic Obese and Non-Diabetic Obese Patients

Diyabetik Obez ve Diyabetik Olmayan Obez Hastalarda Vaspin, Visfatin ve Kemerin Gen Polimorfizimlerinin İncelenmesi

Fikret Kaan ORAN[1] [iD], Arezoo GHEYBI[1] [iD], Faruk CELIK[1] [iD], Sermin DURAK[1] [iD], Zeliha DOGAN[2] [iD], Saadet Busra AKSOYER SEZGIN[3] [iD], Cem BASARAN[4] [iD], Osman FAZLIOGULLARI[5] [iD], Nur Gokce CETINER[1] [iD], Sakir Umit ZEYBEK[1] [iD]

## ABSTRACT

Diabetes mellitus and obesity are very similar in terms of pathogenesis and pathophysiology. Most obese patients have adipose tissue dysfunction caused by genetic and environmental factors. Adipose tissue is the source of adipokines secreted from adipocytes. Vaspin (visceral adşpose tissue-derived serine protease inhibitor), Visfatin (pre-B cell enhancing factor) and Chemerin are adipokines discovered in recent years. The study consists of 43 non-diabetic obese and 51 diabetic obese individuals. The PCR-RFLP method and agarose gel electrophoresis techniques were used to detect gene polymorphisms of Chemerin rs17173608, Vaspin rs2236242 and Visfatin rs2110385 from DNA samples. In our study, when diabetic obese and non-diabetic obese patient groups were examined in terms of Vaspin gene polymorphism, statistically significant results were obtained (22% → 7%, p=0.048, respectively). The distribution of Chemerin or Visfatin gene variants were not different in study groups (p>0.05). Our results indicate that Chemerin rs17173608 and Visfatin rs2110385 gene polymorphisms were not risk factors for development of diabetes in obese individuals, however, Vaspin rs2236242 gene polymorphism may be a contributory risk of development of diabetes in obese individuals.

**Keywords:** Diabetes, Obesity, Vaspin, Chemerin, Visfatin, Polymorphism

## ÖZ

Diabetes mellitus (DM) ve obezite, patogenez ve patofizyoloji açısından çok benzerdir. Obezite hastalarının çoğunda, genetik ve çevresel faktörlerin yol açtığı adipoz doku fonksiyon bozukluğu bulunur. Adipoz doku, adipositlerden salgılanan mediyatörlerin (adipokinler) kaynağı olup Vaspin (visceral adipose tissue–derived serine protease inhibitor), Visfatin (pre-B cell enhancing faktör) ve Kemerin son yıllarda keşfedilen adipokinlerdir. Çalışmamıza 43 Diyabetik obez ve 51 Diyabetik olmayan obez hastalığı tanısı konulmuş toplam 95 hasta dahil edilmiştir. Hasta grubunun 10ml'lik kan örnekleri EDTA'lı tüplere toplanmıştır. DNA örneklerinden Kemerin rs17173608, Vaspin rs2236242 ve Visfatin rs2110385 polimorfizmlerini saptamak için PCR-RFLP method ve agaroz jel elektroforezi teknikleri kullanılmıştır. Çalışma grubumuz Vaspin gen polimorfizimi açısından incelendiğinde istatistiksel olarak anlamlı sonuçlar tespit edilmiş olup (sırasıyla %22 → %7, p=0.048) Kemerin ve Visfatin gen varyantlarının dağılımı açısından istatistiksel olarak anlamlı bulunamamışıtr (p>0.05). Sonuç olarak Kemerin rs17173608 ve Visfatin rs2110385 gen polimorfizmlerinin obez bireylerde diyabet gelişimi için risk faktörü olmadığını, ancak Vaspin rs2236242 gen polimorfizminin obez bireylerde diyabet gelişimi açısından bir risk faktörü olabileceği gösterilmiştir.

**Anahtar Kelimer:** Diyabet, Obezite, Vaspin, Kemerin, Visfatin, Polimorfizm

Sermin DURAK (✉)

*Istanbul University, Aziz Sancar Institute of Experimental Medicine, Department of Molecular Medicine, Vakif Gureba Cad., Sehremini-Fatih 34093 Istanbul, Turkey.*
*e-mail: sermingad@gmail.com*

[1] *Department of Molecular Medicine, Aziz Sancar Institute of Experimental Medicine, Istanbul University, Istanbul, Turkey.*

[2] *Altınbaş University, Vocational School of Health Services, Istanbul, Turkey.*

[3] *Biruni University, Vocational School, Istanbul, Turkey.*

[4] *Biruni University, Faculty of Medicine, Department of Medical Biochemistry, Istanbul, Turkey.*

[5] *Altınbaş University, Vocational School of Health Services, Istanbul, Turkey.*

## INTRODUCTION

In recent years, the prevalence of obesity has been increasing rapidly around the world and poses a major global health problem. The incidence of obesity is increasing day by day in our country, as in other countries of the world.

Worldwide obesity has increased tripled since 1975 and nearly 13% of the world's adult individuals (11% of men and 15% of women) were obese in 2016 [1]. According to the preliminary study report of "Turkey Nutrition and Health Survey-2010" conducted by Turkey Ministry of Health, the prevalence of obesity in Turkey was found 20.5% in men, 41.0% in women and 30.3% in total [2]. Physical inactivity due to increasing sedentary life, genetic and environmental factors, insulin resistance and adipose tissue dysfunction are observed in most obese individuals [3]. There are many diseases associated with obesity such as Type 2 Diabetes Mellitus (T2DM), musculoskeletal disorders, cardiovascular diseases, metabolic syndrome, and some cancer [1]. Obesity, on the other hand, is one of the most important risk factors in T2DM etiology. T2DM is characterized inefficient insulin secretion from langerhans beta cells in pancreatic islets that known as insulin resistance [4]. The resulting decrease in glucose uptake, mainly due to the reduced effect of insulin in target tissues such as adipose tissue, skeletal muscle and liver cells [5].

Adipose tissue is an endocrine organ and is responsible for the secretion of cytokines called adipocytokines or adipokines. These cytokines play important roles in carbohydrate and fat metabolism in the regulation of the hunger and satiety cycle. Adipokines are effective in the development of insulin resistance associated with obesity and also play a role in a wide variety of physiological and pathological processes, including immunity and inflammation, by showing endocrine, paracrine, autocrine and juxtracrine effects [6]. Excess adipose tissue lead to secretion of adipocytes and the development of chronic inflammation in obese individuals. There is increased expression of adhesion molecules along with adipokine-induced chemokines stimulate macrophage secretion from adipose tissue. İnsulin resistance development because of local inflammation [7]. Various pro-inflammatory peptides secreted by macrophages of adipose tissue in obesity; Tumor necrosis factor – α (TNF-α) and interleukin 6 (IL-6) are evaluated as adipokine production [8]. These pro-inflammatory adipokines contribute a lot to the "low-grade inflammatory state" of obese cases and cause cardiovascular complications and autoimmune inflammatory diseases. In adipose tissue, also anti-inflammatory factors such as interleukin 1-receptor antagonist (IL-1RA) and IL-10 are produced which are significantly increased in obese patients [9]. Data from studies on mice suggest that this endogenous antagonist exerts both central and peripheral effects, thus increasing adipogenesis and leptin resistance. Also, the ratio IL-1RA/IL-1 favors IL-1RA and is the best IL-1RA inducer in interferon (IFN) – β WAT. In addition, it cannot modulate leptin or adiponectin in IFN-β WAT [8]. New ones are joining the adipokine family recently, and the most known ones are leptin, adiponectin, resistin, TNF-α, interleukin-6, apelin, acylation stimulating protein, plasminogen activator inhibitor factor-1 and renin angiotensin system proteins. The adipokines discovered in recent years are *Vaspin* (visceral adipose tissue – derived serine protease inhibitor), *Visfatin* (pre-B cell enhancing factor), and *Chemerin* [10]. *Vaspin* is a serine protease inhibitor produced in visceral adipose tissue. It has been suggested that *Vaspin* induction by adipose tissue may create a compensatory mechanism in response to obesity and inflammatory complications of obesity. *Vaspin* levels were found to increase significantly in obese and insulin resistant mice [11]. *Vaspin* can be considered as a new link between obesity and associated metabolic disorders, such as glucose intolerance [12]. *Visfatin* is an insulin-mimicking adipokine discovered in liver, skeletal muscle and bone marrow as a growth factor for B lymphocyte precursors. Circulating *Visfatin* level is closely associated with white adipocyte tissue accumulation. *Visfatin* mRNA levels increase during adipocyte differentiation and *Visfatin* synthesis is regulated by various factors such as glucocorticoids, TNF, IL-6 and growth hormone [13]. *Chemerin*, also called retinoic acid receptor responsive protein 2 (RARRES2), is a new adipokine and its biological functions are not yet clear. *Chemerin* is a protein secreted by adipocytes and plays an autocrine / paracrine role in the development and function of adipose tissue. *Chemerin* and its major receptor Chemokine-like receptor 1 (CMKLR1) are secreted in white adipose tissue and increase in obese people and rodents [14,15].

The studies are needed to achieve success in the fight against obesity, adipose tissue and the adipokines produced by them must be well known. Moreover, we think that the impaired adipokine production observed in obesity contributes to diabetes pathogenesis. We believe that there are differences in polymorphism in the adipokine genes in the pathophysiology of these two diseases. Our aim was to determine the differences in gene variations of adipose tissue adipokines of *Chemerin rs17173608, Vaspin rs2236242 and Visfatin rs2110385* in diabetic obese and non-diabetic obese patients.

## MATERIAL AND METHODS

### Participants

In this study, two separate sample groups were employed. The participants in the study were selected from 43 non-diabetic obese patients and 51 diabetic obese patients followed by Medicana Bahçelievler Hospital Cardiovascular Surgery Clinic.

All participants in the study provided their written consent prior to the study. Body Mass Index (BMI=kg/m$^2$) measurements of the patients were selected according to the age-related obesity limit. The weight, height and body composition measurements of the groups that were included in the study were recorded. The biochemical parameters, such as Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Blood Urea Nitrogen (BUN), keratin, platelet (PLT), sedimentation, urea, White Blood Cell (WBC), HbA1c, Low Density Lipoprotein-Cholesterol (LDL-Cholesterol), High Density Lipoprotein-Cholesterol (HDL-Cholesterol), Very Low-Density Lipoprotein (VLDL) were examined.

### DNA isolation and PCR-RFLP

The genomic DNA was obtained by using salting-out technique [16]. The polymorphic genotyping was determined by using polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP) and Agarose Gel Electrophoresis Techniques as previously reported [17,18,19]. The *Chemerin, Vaspin* and *Visfatin gene* polymorphisms were performed by using Alu1 endonuclease restriction enzyme.

### Statistical Analysis

The SPSS 21.0 software was used for statistical analysis. The statistical significance was accepted as $p<0.05$. Evaluation of relative risk was determined by calculating confidence intervals (CI) and odds ratio (OR). Student's t-test was used to determine the difference in biochemical parameters as mean ± standard deviation. The Chi-Square ($\chi^2$) and Fisher's Exact test were used to compare of genotypes distributions data as percentage (%) and determine their effects on biochemical activity.

## RESULTS

The present study includes 43 non-diabetes obese and 51 diabetes obese patients, and the study groups have a similar distribution of sex *(p > 0.05)*. The clinical data of the study groups were shown in Table 1. The cases participating in the study were evaluated in terms of biochemical and somatic parameters, such as age, height, weight, BMI, HbA1c, LDL, HDL, and low VLDL. Among these, it was determined that age caused significant differences between diabetic and non-diabetic groups (Table 1).

**Table 1.** Clinical data of diabetic and non-diabetic obese patient groups

| Clinical par. | Non-Diabetic obese (n=43) | Diabetic obese (n=51) | *p value* |
|---|---|---|---|
| Age | 64.74±5.97 | 61.47 ±9.38 | ***0.013**[*]* |
| BMI | 31.60±3.32 | 31.93 ±3.27 | 0.687 |
| LDL | 118±44.59 | 120.85 ±36.31 | 0.733 |
| AST | 28.16±17.55 | 31.67 ±17.19 | 0.119 |
| ALT | 27.21±14.98 | 35.00 ± 27.06 | 0.133 |
| BUN | 20.86±9.97 | 20.14 ±10.55 | 0.586 |
| Creatinine | 1.67±4.7 | 1.10±1.15 | 0.969 |
| PLT | 271.8±66.01 | 272.08±61.95 | 0.983 |
| ESR | 36.60±22.24 | 32.61±23.27 | 0.270 |
| Urea | 42.45±17.94 | 43.11±20.82 | 0.734 |
| WBC | 8.13±2.37 | 7.60±1.99 | 0.247 |
| Weight | 81.29±11.17 | 85.23±12.54 | 0.087 |
| HDL | 39.92±9.44 | 38.17±9.44 | 0.490 |
| VLDL | 48.88±45.37 | 38.79±9.44 | 0.642 |

*The difference between the groups was analyzed with student's t-test and Chi square (×2) test. The bold values p<0.05 indicate statistical significance (Mean ± Standard Deviation).*

The distribution of genotype of *Chemerin, Vaspin* and *Visfatin* polymorphisms in the study groups was shown in Table 2. In our study, which was conducted with diabetic and non-diabetic obese individuals, *Vaspin TT* genotype was found to be statistically significant between the two groups *(22% → 7%, p=0.048,* respectively*)*. However, no significant differences were detected between the groups in *Chemerin* and *Visfatin* gene polymorphism *(p>0.05)* (Table 2).

**Table 2.** Genotypic distribution of *Chemerin*, *Vaspin* and *Visfatin* polymorphisms in diabetic and non-diabetic obese patients

| Genotypes | Non – Diabetic obese (n=43, %) | Diabetic obese (n=51, %) | p value |
|---|---|---|---|
| Chemerin rs: 17173608 | | | |
| TT (w) | 21 (49%) | 20 (39%) | |
| TA | 17 (39%) | 22 (43%) | 0.46 |
| AA | 5 (12%) | 9 (18%) | |
| Vaspin rs: 2236242 | | | |
| GG(w) | 19 (44%) | 15 (29%) | |
| GT | 21 (49%) | 25 (49%) | **0.048*** |
| TT | 3 (7%) | 11 (22%) | |
| Visfatin rs: 2110385 | | | |
| TT (w) | 12 (28%) | 8 (16%) | |
| TG | 18 (42%) | 19 (37%) | 0.096 |
| GG | 13 (30%) | 24 (47%) | |

*The differences between the groups were analyzed with the Chi-Square ($X^2$) test for values with percentage (%). The bold values p<0.05 indicate statistical significance.*

Moreover, the clinical parameters and possible diabetes risk factors (age, AST, LDL and BMI) were also not associated with homozygote mutation genotype *Chemerin (p>0.05)* (Table 3). However, when evaluated in terms of age in the study of homozygote mutation genotype in *Vaspin and Visfatin* were found significant *(p=0.032, OR:1.065, CI:1.005-1.128; p=0.42, OR:1.061, CI: 1.002-1.124;* respectively*)*. Therefore, the homozygote mutant polymorphism in *Vaspin and Visfatin* accompanied by increased age might increase the diabetes risk factor in obese patients (Table 3). On the other hand, AST, LDL and BMI show no significant differences in *Vaspin and Visfatin* homozygote mutation genotypes *(p>0.05)* (Table 3).

**Table 3.** The evaluation of *Chemerin Vaspin* and *Visfatin* homozygote polymorphism genotypes in terms of diabetic risk factors in diabetic obese and non-diabetic patient groups.

| Genotypes and risk factors | OR | 95% CI | p value |
|---|---|---|---|
| *Chemerin (AA)* | 0.520 | 0.143-1.889 | *0.320* |
| Age | 1.052 | 0.995-1.112 | *0.07* |
| BMI | 0.985 | 0.865-1.122 | *0.820* |
| LDL | 1.001 | 0.990-1.012 | *0.892* |
| AST | 0.986 | 0.961-1.01 | *0.261* |
| *Vaspin (TT)* | 0.234 | 0.058-0.951 | *0.042* |
| Age | 1.065 | 1.005-1.128 | ***0.032*** |
| BMI | 1.008 | 0.880-1.115 | *0.910* |
| LDL | 1.002 | 0.991-1.013 | *0.753* |
| AST | 0.989 | 0.964-1.014 | *0.386* |
| *Visfatin (GG)* | 0.411 | 0.160-1.005 | *0.065* |
| Age | 1.061 | 1.002-1.124 | ***0.042**** |
| BMI | 1.020 | 0.889-1.70 | *0.782* |
| LDL | 1.002 | 0.991-1.014 | *0.664* |
| AST | 0.990 | 0.965-1.055 | *0.437* |

*The differences between the groups were analyzed with Chi-Square ($X^2$). The bold values p<0.05 indicate statistical significance (Mean ± Standard Deviation).*

## DISCUSSION

Diabetes mellitus and obesity are very similar in terms of pathogenesis and pathophysiology. Most obesity patients have adipose tissue dysfunction caused by genetic and environmental factors. Adipose tissue is the source of adipokines secreted from adipocytes. In recent years, *Vaspin, Visfatin, Chemerin* are ones of the important adipokines.

The differences in polymorphisms in adipokine genes are thought to cause the pathophysiology of T2DM and obesity [20]. In this study, *Chemerin, Vaspin and Visfatin* gene were determined and the obtained findings were compared with clinical parameters in diabetic obese and non-diabetic obese patients.

Nora klö et al. (2006) reported that the *Vaspin* gene expression was found statistically significant in T2DM (p<0.0001) [21]. In the present study, statistically significant results were obtained in *Vaspin* gene polymorphism between the diabetic obese and non-diabetic obese patient groups (*22% → 7%, p=0.048, respectively*). Previous studies suggested the *Vaspin* gene might be an important marker in development both obesity and impaired insulin resistance [22,9]. Moreover, the *Vaspin* gene could be considered of T2DM in obese individuals. Feng et al. conducted in the study, the T2DM with obese patients, it was reported that there was a statistically significant relationship between serum HbA1c level and *Vaspin* level. In the same study, it was reported that there was a significant relationship between BMI and age in the control group [23]. In addition, Francisca Lago et al. reported that the age and gender regulate *Vaspin* gene expression and also *Vaspin* serum levels are associated with biochemical parameters [6]. In our study, we found statistically significant relationship between homozygous mutation in the *Vaspin* gene and the age parameter (*p=0.032, OR:1.065, CI:1.005-1.128*). The presence of homozygous mutation in the *Vaspin* gene risk of developing diabetes increases with age factor. However, there was no significant difference between *Vaspin* gene and genders in our study. On the other hand, Byung Son Youn et al. reported that the *Vaspin* serum level showed differences according to gender. It was observed that *Vaspin* serum levels in women increased approximately 2.5-fold compared to men [22].

In the literature, the results of studies examining the relationship between diabetic obese and *Chemerin* gene polymorphism are inadequate. Therefore, we have examined *Chemerin* gene polymorphism in study groups. *Chemerin* secreted from white adipose tissue is a signal

molecule with a variety of paracrine and autocrine effects and a new adipokine that regulated adipogenesis, metabolic homeostasis, glucose metabolism, inflammation, and many physiological processes in the cell. Weigert et al. showed that *Chemerin* serum level was similar in T2DM and obese individuals but was significantly increased in both cohorts compared to healthy individuals [24]. In the present study, we found no significant association between *Chemerin* gene polymorphism and study groups (p>0.05). In addition, the presence of homozygous mutation in *Chemerin* gene was not found to be statistically significant when examined in terms of somatic and biochemical parameters (such as age, BMI, LDL and AST).

The third step of our study is to examine the *Visfatin* gene polymorphism in study groups. The *Visfatin* are essentially produced by macrophages and play a major role in regulation of glucose transport and triacylglycerol (TAG) synthesis and was thus originally proposed to be insulin mimetic. In addition, *Visfatin* is recognized as a new proinflammatory adipocytokine and presumed to play an important role in inflammatory process of T2DM [25]. Sreedharan et. al. reported that the *Visfatin* serum levels were significantly higher in diabetic patients compared with nondiabetic group *(11.4 ± 5.9 → 9.8 ± 4.3 ng/mL, p=.008).* Moreover, within the non-diabetic group, the *Visfatin* serum levels were significantly higher in obese individual compared with non-obese individual (10.4 ± 4.7 → 9.0 ± 3.8 *ng/mL*, p=.048) and the significance was maintained in terms of age and sex (OR:1.086; CI:1.003-1.176; p=.042) [26].

In our study, a statistically significant difference could not be found between diabetic obese and non-diabetic obese patients in regards of *Visfatin* gene polymorphism. However, in our study, we can say that the development of homozygous mutation in the *Visfatin* gene is statistically significant in terms of age parameter and the risk of diabetes may be higher in obese cases as the age gets older (p<0.042). In our study, statistically significant results were not obtained in terms of biochemical parameters in the presence of homozygous mutation in the *Visfatin* gene.

Consequently, the statistically significant results were found in terms of *Vaspin* gene polymorphism in diabetic obese and non-diabetic obese patient groups. Accordingly, the combined effect of increasing age in obese individuals and the presence of homozygous mutations in the *Vaspin* gene may be a contributory risk of development of diabetes in obese individuals. Our other two genes, *Chemerin* and

*Visfatin* gene polymorphism, were not statistically significant difference between the groups resulting from low number of patients. The main problem of our study is that the genes and biochemical values are not statistically significant due to small number of patients. As a result, in order to understand the relationship between the development of T2DM in obese patients, *Chemerin rs17173608, Vaspin rs2236242 and Visfatin rs2110385* polymorphisms should be developed in larger groups and with broader clinical data. We think that it will contribute to the literature as it constitutes the first data analyzed in terms of diabetes development among obese patients in Turkish society.

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