Received: 16-10-2022 | **Accepted:** 20-10-2022
DOI: 10.54005/geneltip.1190070

ORIGINAL ARTICLE

Association of Celiac Disease and Plasminogen Activator Inhibitor-1 Polymorphism

Çölyak Hastalığı ve Plazminojen Aktivatör İnhibitör-1 Polimorfizminin İlişkisi

[1]Deniz Ağırbaşlı, [2]Minenur Kalyoncu, [3]Nur Ramoğlu, [4]Zuhal Çalışkan

[1]Istanbul University-Cerrahpasa, Cerrahpasa Medical Faculty, Department of Medical Genetics, İstanbul
[2]Dokuz Eylul University, Izmir International Biomedicine and Genome Institute, Department of Biomedicine and Health Technologies, Izmir
[3]Acıbadem Mehmet Ali Aydınlar University Faculty of Medicine, Department of General Surgery, Istanbul
[4]University of Health Sciences, Istanbul Umraniye Medical Center, Department of Gastroenterology, Istanbul

Correspondence

Deniz Ağırbaşlı, Istanbul University-Cerrahpasa, Cerrahpasa Medical Faculty, Department of Medical Genetics, İstanbul/TÜRKİYE

E-Mail: deniz.agirbasli@iuc.edu.tr

How to cite ?

Ağırbaşlı D. , Kalyoncu M. , Ramoğlu N. , Çalışkan Z. Association of Celiac Disease and Plasminogen Activator Inhibitor-1 Polymorphism. Genel Tıp Dergisi. 2022; 32(5): 601-604

ABSTRACT

Objective: Celiac disease (CD) is an autoimmune disease. Although susceptibility to thrombosis in celiac patients has been reported in case studies, the cause is not yet known. PAI-1 inhibits fibrinolysis. In this study, the association between celiac disease and PAI-1 4G/5G polymorphism was investigated among celiac patients whose disease were confirmed by intestinal biopsy and healthy controls.
Methods: Biopsy-diagnosed celiac patients (n=56) and healthy controls (n=52) were included in the study. PCR-RFLP method was used for PAI-1 genotyping and the results were compared according to statistical significance.
Results: The PAI-1 4G allele associated with thrombotic risk and inflammation was found to be higher than the 5G allele in cases (0.63 and 0.36, respectively, p=0.054). When the age at diagnosis was compared according to the PAI-1 variants, no significant difference was found (p=0.3). Although the genotype frequencies were similar in 4G/4G patients and controls, the 5G/5G genotype, known as the protective genotype, was found to be higher in controls (35% and 18%).
Conclusion: In our study, the 4G allele of PAI-1, which plays a role in the susceptibility to thrombosis, was observed more frequently in celiac patients compared to the control group. Studying genetic markers of thrombosis in celiac patients is important for individual prophylaxis.

Keywords: Celiac disease; Plasminogen Activator Inhibitor 1; Polymorphism; Thrombosis

ÖZ

Amaç: Çölyak hastalığı (ÇH) otoimmün bir hastalıktır. Çölyak hastalarında tromboza yatkınlık vaka çalışmalarında bildirilmiş olmasına rağmen nedeni henüz bilinmemektedir. Plazminojen aktivatör inhibitörü-1 (PAI-1), fibrinolizi inhibe eder. Bu çalışmada biyopsi ile çölyak tanısı konulmuş kişiler ve sağlıklı kontrollerde çölyak hastalığı ile PAI-1 4G/5G polimorfizmi arasındaki ilişki araştırılmıştır.
Yöntemler: Çalışma için 56 biyopsi ile tanı konulmuş çölyak hastası ve 52 sağlıklı kontrol seçilmiştir. PAI-1 genotiplendirmesi PZR-RFLP metodu ile yapılmış, sonuçlar istatistiksel anlamlılığa göre karşılaştırılmıştır.
Bulgular: Trombotik risk ve enflamasyonla ilişkilendirilen PAI-1 4G aleli vakalarda 5G aleline oranla yüksek bulundu (sırasıyla 0.63 ve 0.36, p=0.054). PAI-1 varyantlarına göre tanı yaşları kıyaslandığında anlamlı bir fark bulunmadı (p=0.3). Genotip olarak 4G/4G hastalar ve kontrollerde benzer olsa da koruyucu genotip olarak bilinen 5G/5G genotipi kontrollerde hastalara kıyasla yüksek bulunmuştur (35% ve 18%).
Sonuç: Çalışmamızda çölyak hastalarında kontrol guruba göre PAI-1'in tromboza yatkınlıkta rol oynayan 4G aleli daha sık gözlenmiştir. Çölyak hastalarında tromboz genetik belirteçlerinin çalışılması bireysel proflaksi açısından önem taşımaktadır.

Anahtar Kelimeler: Çölyak hastalığı; Plazminojen aktivatör inhibitörü 1; Polimorfizm; Tromboz

Introduction

Celiac Disease is a multifactorial disease in which both environmental (gluten) and genetic (HLA and non-HLA genes) factors play a role. An abnormal immune response to gluten, causes inflammation and injury to the small intestine mucosa (1). The world prevalence of celiac disease is 0.5-1.0% (2). People with autoimmune diseases and diabetes, and relatives of Celiac patients are at high risk for the disease. Gastrointestinal complaints such as diarrhea, steatorrhea, and weight loss due to malabsorption is common. Anemia, osteoporosis, dermatitis, herpetiformis, neurological problems and dental enamel hypoplasia may accompany as atypical symptoms. Celiac disease is diagnosed by biopsy and by the presence of a genetic factor predisposing to positive anti-tissue transglutaminase and/or antiendomysial antibodies (HLA) DQ2/8 antigen (3). Although a predisposition to thrombosis in celiac patients has been reported in case studies, the cause is not yet known (4). To date, hyperhomocysteinemia, B12 deficiency, endothelial or platelet dysfunction have been investigated for predisposition to thrombosis (5). The diversity of hypercoagulation-related diseases associated with celiac disease indicates that the etiology of hypercoagulability in celiac disease is multifactorial, and that thromboembolism prophylaxis should be regulated according to the variety of predisposing factors. Plasminogen activator inhibitor-1 (PAI-1) is found in the alpha granules of platelets and in plasma. It inhibits fibrinolysis by inhibiting the conversion of plasminogen to plasmin. At the same time, as a serine protease, it has an important role in many cellular

activities such as wound healing, organ fibrosis, aging, autophagy, digestion, immune system and tumor metastasis (6-9).

Although many variants have been reported in the *PAI-1* gene, the 1bp ins/del 4G/5G polymorphism in the transcription start region affects PAI-1 levels. Although both alleles bind to the transcriptional activator, 5G also binds to the transcriptional repressor. Thus, the 4G allele increases the transcription of PAI-1, especially in the homozygous form (10). The aim of the study is to compare *PAI-1* 4G/5G polymorphism genotypes and allele frequency in individuals diagnosed with celiac and healthy controls.

Materials and Methods

Study group:

The study included 56 patients who applied to the gastroenterology outpatient clinic of Ümraniye State Hospital between July 2017 and December 2018 and were diagnosed with celiac disease as a result of small bowel biopsy, and 52 healthy controls without any gastrointestinal complaints. The study was performed according to the Helsinki declaration and was approved by the Institutional Review Board with the decision number 2017-3/36. Written informed consent was obtained from all participants. Exclusion criteria from the study were defined as people who read the consent form and refused to participate in the study, people with an autoimmune disease before the diagnosis of celiac disease, cancer patients, celiac patients with infectious diseases (eg. hepatitis B). Subjects were asked to fill out a questionnaire about their demographic information, dietary habits, physical activity, family history in terms of celiac and cardiovascular diseases.

Peripheral blood collection and DNA isolation:

All genetic analysis were done in Acibadem Mehmet Ali Aydınlar University Research Laboratory. Two cc of blood from the peripheral vein (Median cubital vein) from the patients and controls was taken into EDTA tubes and stored at 4°C. Commercial kit were used for DNA isolation according to the protocol described by manufacturers (NucleoSpin® Tissue XS- Macherey Nagel, Düren, Germany). After DNA isolation, DNA purity was measured with Varioskan Flash® Thermo Scientific (Waltham, MA, USA). DNA isolation was considered successful, if A260/280 absorbance ratio was 1.7-2.0 for all samples. DNA amounts were calculated as 40-60 ng/µl.

PCR (Polymerase Chain Reaction) and RFLP (Restriction Fragment Length Polymorphism) experiments

DNA was amplified by PCR with appropriate primers. Genetic variants were analyzed using the RFLP method. Primers required to amplify the region with the *PAI-1* 4G/5G polymorphism (rs1799889) was

F: 5'- CACAGAGAGAGTCTGGCCACGT-3',

R: 5'-CCAACAGAGGACTCTTGGTCT-3' (11). MyTaq™ Mix (Meridan Bioscience, Memphis, TN, USA) was used for the PCR Reaction. Samples were amplified on the BIO-RAD T100 Thermal Cycler (BIO-RAD Laboratories, CA, USA). PCR cycles were determined as (94°C, 3', (94°C, 30'', 53°C 30'', 68°C 20'')x 35 cycles, 68°C 2', 4°C ∞). PCR products were analyzed by 2% agarose (Low EEO) gel electrophoresis and visualized on the ChemiDoc MP Imaging System (BIO-RAD, CA, USA).

The restriction enzyme *BslI* (New England Biolabs®, *Inc. MA,* USA) was used to identify the *PAI-1* variants in the amplified region. RFLP is based on the principle that variations in the DNA sequence can alter restriction enzyme cleavage patterns. Since there was no enzyme cut in the region of 4G/4G genotype, the product was seen as a single band of 100 bp, the enzyme cuts in 5G/5G genotype, and it was observed as 2 bands of 77 bp and 23 bp. In heterozygous 4G/5G genotype, 3 bands (100,77,23) were observed for both cleaved and uncleaved fragments (Figure 1)

Statistical analysis

The SPSS program (version 20.0 for Windows, SPSS Inc. Chicago, IL) was used to evaluate the statistical data. One Sample Kolmogorov-Smirnov test was used to analyze the normality of the data. Parametric tests were used for normally distributed data and otherwise non-parametric tests were used. Continuous variables with normal distribution were expressed as mean ± standard deviation, and variables in non-parametric distribution were expressed as median and interquartile range (IQR). Categorical variables were expressed in percentiles (%). Values with $p< 0.05$ were considered statistically significant.

Results

The study included 56 cases and 52 controls. Gender distribution was different between the cases and controls. Of the case group (n=56), 11% were male, 89% were female; 39% of the control group (n=52) were male and 61% were female ($p<0.001$). The mean ages of the case and control groups were 38±11 and 22±3 ($p=0.001$), respectively. There was no statistically significant difference in body mass index (BMI) between cases and controls (24.5±4.7, 23±4.5 ($p=0.3$)). The mean age at which the patients were diagnosed was 32.8 years. In the survey, 11.5% of the patients stated that they started a diet after the diagnosis of Celiac disease.

There was no statistically significant differences in the family history of cases and controls. Cardiovascular disease was present in 7.6% of patients and 12.5% of controls ($p=0.375$). Patients exercised less than the controls. The questionnaire results displayed that walking 2 km at least 3 times a week as exercise was present in 12% of patients and 69% of controls ($p<0.001$). Genotype frequencies of cases and controls were as

shown in Table 1. Allele frequencies for 4G and 5G were as shown in Table.2.

Allele frequencies were compatible with Hardy Weinberg equilibrium (X2 =1.5, p=0.2) When the allele frequencies were compared between the cases and controls, the 4G allele was more frequently observed in cases versus controls (0.63 and 0.37, respectively; p=0.054). The 5G/5G genotype, known as the protective genotype, was found to be more frequent in controls than the cases (35% and 18%). No statistically significant difference was found in the age at diagnosis between the genotype groups in celiac patients.

Table 1. Genotype frequencies according to PAI-1 variants in cases and controls

PAI-1 4G/5G	4G/4G	4G/5G	5G/5G
Cases n(%)	24(43)	22(39)	10(18)
Controls n(%)	23(44)	11(21)	18(35)

Table 2. Frequency of 4G and 5G alleles in patients and controls

	4G	5G
Cases	0.63	0.37
Controls	0.55	0.45

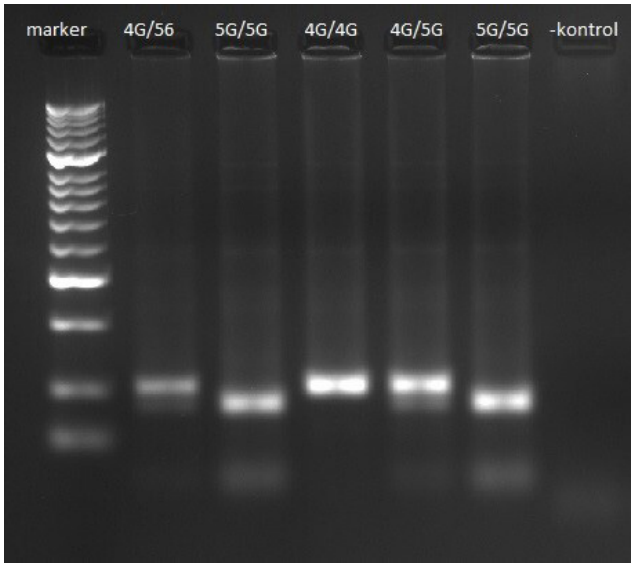

Figure 1. The detection of *PAI-1* variants by *BslI* enzyme restriction of PCR amplified DNA on 2% agarose gel (4G/4G=100bp, 4G/5G=100bp,77bp,23 bp, 5G/5G= 77bp,23 bp)

Discussion

Celiac disease is a complex phenotype with vascular effects. Thromboembolic events in celiac patients are included in the literature. The pathophysiology in Celiac disease involves thrombosis, and inflammation (12). Plasminogen activator inhibitor 1 (PAI-1) is a serpin inhibitor of the plasminogen activators urokinase-type plasminogen activator (uPA) and tissue plasminogen activator. PAI-1 plays a role in thrombosis and inflammation, and its relationship with celiac disease is the subject of experimental and clinical research. PAI-1 is involved in a wide range of biological and pathological processes such as wound healing and angiogenesis, extracellular matrix remodeling.

In the literature, PAI-1 blood levels have also been found to have an effect on autonomic nervous system disorder seen in Celiac patients (15), and PAI-1 levels have been associated with inflammatory changes in the colonic mucosa (16). Inflammatory changes seen in celiac disease question the role of PAI-1 in this disease.

The *PAI-1* 4G/5G polymorphism results from a single nucleotide deletion/insertion (4G/5G). Depending on the increase in PAI-1 concentration in the 4G/4G genotype, fibrinolytic activity is impaired and susceptibility to thrombotic events increases. Studies report that 4G allele of the polymorphism increases the tendency to inflammation and clotting in coronary patients.

Celiac disease is in the class of complex diseases in which genetic and environmental factors play a role. The aim of our study is to evaluate the effects of *PAI-1* 4G/5G polymorphism, which affects PAI-1 levels, on the pathogenesis of Celiac.

According to our results, the *PAI-1* 4G/4G variant was not associated with age at diagnosis in 56 celiac patients. All cases were diagnosed with biopsy and 52 healthy controls were included for comparison. The study findings indicate that 4G allele frequency is higher in Celiac cases compared to the controls. The study findings can explain the increased thrombotic events in Celiac cases. There are limited studies in the literature on this subject. The limitation of the study is that it was studied with a small number of cases and controls. It is important to expand studies in larger cohorts to include other thrombotic factors. In addition, polymorphism studies alone are not sufficient to understand the complex diseases. In addition to polymorphism studies, studies examining gene expression at the transcription level are required to assess the functional effects.

The expressions of cells affected by different diseases can be compared with the gene expressions of healthy cells and used in disease diagnosis and target drug design. Investigation of genetic susceptibility to thrombosis is recommended for thromboembolic prophylaxis in adult celiac patients (17). For this reason, the importance of thrombosis genetic markers in celiac patients is a current research topic.

References

1.Farrell RJ, Kelly CP. Celiac sprue. N Engl J Med 2002;346(03):180–88.

2.Gujral N, Freeman HJ, Thomson ABR. Celiac disease: Prevalence, diagnosis, pathogenesis and treatment World J Gastroenterol. 2012;18(42):6036–59.

3.ESPGN Group: Revised criteria for diagnosis of coeliac disease. Report of Working Group of European Society of Paediatric Gastroenterology and Nutrition. Arch Dis Child 1990;65:909-11

4.Ghannouchi Jaafoura N, Atig A, Bouker A, Alaoua O, Ben Jazia E, Khalifa M, Bahri F. Intracardiac thrombosis during celiac disease. J Mal Vasc. 2014;39(3):203-6.

5.Lerner A, Blank M. Hypercoagulability in celiac disease--an update. Autoimmun Rev. 2014;13(11):1138-41.

6.Agirbasli M. Pivotal role of plasminogen-activator inhibitor 1 in vascular disease. Int J Clin Pract 2005;59(1):102-6.

7.Flevaris P, Vaughan D.The Role of Plasminogen Activator Inhibitor Type-1 in Fibrosis. Semin Thromb Hemost. 2017;43(2):169-77.

8.Wang ZH, Ren WY, Zhu L, Hu LJ. Plasminogen activator inhibitor-1 regulates LPS induced inflammation in rat macrophages through autophagy activation. Sci World J. 2014;2014:189168.

9.Pham BT, van Haaften WT, Oosterhuis D, Nieken J, de Graaf IA, Olinga P. Precision-cut rat, mouse, and human intestinal slices as novel models for the early-onset of intestinal fibrosis. Physiol Rep. 2015;3(4). pii: e12323

10.Eriksson P, Kallin B, van 't Hooft F M, Båvenholm P, and Hamsten A. Allele-specific increase in basal transcription of the plasminogen-activator inhibitor 1 gene is associated with myocardial infarction. Proc Natl Acad Sci U S A. 1995;92(6):1851–55.

11.Rossaak JI, Van Rij AM, Jones GT, Harris EL. Association of the 4G/5G polymorphism in the promoter region of the plasminogen activator inhibitor-1 with abdominal aortic aneurysms. J Vasc Surg 2000;31:1026–32.

12.Ludvigsson JF, Welander A, Lassila R, Ekbom A, Montgomery SM. Risk of thromboembolism in 14,000 individuals with coeliac disease. Br J Haematol 2007;139(01):121–7.

13.Ciacci C, Tortora R, Scudiero O, Di Fiore R, Salvatore F, Castaldo G. Early pregnancy loss in celiac women: The role of genetic markers of thrombophilia. Dig Liver Dis. 2009;41(10):717-20.

14.Picchi A1, Pasqualini P, D'Aiello I, Cortese B, Micheli A, Limbruno U. Acute ST-elevation myocardial infarction in a 15-year-old boy with celiac disease and multifactorial thrombotic risk. Thromb Haemost. 2008 Jun;99(6):1116-8.

15.Zimmerman M, Rolandsson E, Skärstrand H, Pourhamidi K, Gottsäter A, Wollmer P, Rolandsson O, Westergren-Thorsson G, Dahlin LB. Temporal trend of autonomic nerve function and HSP27, MIF and PAI-1 in type 1 diabetes. J Clin Transl Endocrinol. 2017;8:15-21.

16.Grabarczyk E1, Szaflarska-Popławska A, Góralczyk K, Rość D. Tissue plasminogen activator (t-PA) and plasminogen activator inhibitor-1 (PAI-1) in children with ulcerative colitis. Pol Merkur Lekarski. 2006;20(117):326-8.

17.Berthoux E, Fabien N, Chayvialle JA, Ninet J, Durieu I. Adult celiac disease with thrombosis: a case series of seven patients. Role of thrombophilic factors. Rev Med Interne 2011;32(10):600–604.