Original paper

The preliminary association study of osteopontin 707 C/T polymorphism with systemic lupus erythematosus in a Polish population

Beata Kaleta[1], Piotr Mróz[2], Andrzej Górski[1], Jacek Łukaszkiewicz[2], Anna Woźniacka[3], Jarosław Bogaczewicz[3]

[1]Department of Clinical Immunology, Medical University of Warsaw, Warsaw, Poland
[2]Department of Biochemistry and Clinical Chemistry, Medical University of Warsaw, Warsaw, Poland
[3]Department of Dermatology and Venereology, Medical University of Lodz, Lodz, Poland

Adv Dermatol Allergol 2020; XXXVII (2): 190–194
DOI: https://doi.org/10.5114/ada.2019.83499

Abstract

Introduction: Systemic lupus erythematosus (SLE) is a chronic autoimmune disease caused by genetic, environmental, and still unknown factors which lead to deregulation of the immune system. Osteopontin (OPN) is a multifunctional glycoprotein, expressed in various cell types, and found to play key roles in immunity. OPN and variants of the OPN gene are involved in inflammatory conditions, however, their role in SLE are controversial.
Aim: To investigate the frequency of single nucleotide polymorphism (SNP) rs1126616 (707 C/T) variants in the OPN gene and its associations with SLE manifestations in Polish patients.
Material and methods: The study population consisted of 83 SLE patients and 100 gender-, age- and ethnically matched healthy controls. DNA was extracted from whole blood samples using the standard procedure. Genotyping was performed by real-time polymerase chain reaction (RT-PCR). The association between clinical features of SLE and 707 C/T genotypes was determined.
Results: The mutant (CT, TT) genotypes were observed more frequently than the wild-type (CC) genotype in SLE patients compared to controls (p = 0.037). However, no association between 707 C/T variants and SLE clinical manifestations or laboratory parameters was found.
Conclusions: The present data suggest that CT and TT genotypes of OPN 707 C/T SNP are associated with a higher SLE risk, but do not affect the clinical course of the disease in the Polish population.

Key words: gene, osteopontin, polymorphism, systemic lupus erythematosus.

Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease caused by environmental, hormonal and genetic factors which lead to immunological abnormalities [1]. Immune system deregulation leads to production of autoantibodies and cytokines. Autoantibodies form complexes with antigens, which are deposited in organs, causing inflammation and tissue damage [2, 3]. The aetiology of SLE is not fully explained but many studies showed the association between the disease and genes crucial to the immunological response [4]. In the literature, there are reports suggesting that osteopontin (OPN) participates in the pathogenesis of SLE. OPN, also known as early T lymphocyte activation-1 (Eta-1) or SPP-1 (secreted phosphoprotein 1), is a protein secreted by various cell types such as bone cells, neurons and immune cells (B and T lymphocytes, natural killer cells, NKT cells, macrophages, neutrophils, dendritic cells) [5, 6]. OPN may be involved in the pathogenesis of SLE as it was found to regulate cellular immunity, including innate and adaptive components [5, 7–9]. OPN stimulates antibodies production [10–13], regulates macrophages migration, activation, capacity for phagocytosis and nitric oxide production [7, 10, 11] and enhances IL-17 producing Th17 cell responses [9, 12]. Moreover, OPN induces dendritic cells maturation [11] and promotes activation of T lymphocytes [10].

It has been documented that the serum OPN level is elevated in SLE patients and correlates with increased disease activity [14–18]. The expression of OPN is influenced by genetic polymorphisms of its promoter [19]. OPN variants may have a key role in creating a background favouring lymphocyte accumulation and leading

Address for correspondence: Beata Kaleta, Department of Clinical Immunology, Medical University of Warsaw, 59 Nowogrodzka St, 02-006 Warsaw, Poland, phone: +48 600 301 690, e-mail: kaletabeata1@gmail.com
Received: 15.05.2018, **accepted:** 27.09.2018.

to the development of autoimmunity. So far, several polymorphisms in the human OPN gene (4q21-4q25) have been identified [20].

In recent years, OPN and its gene polymorphisms have been associated with susceptibility to autoimmune diseases, including multiple sclerosis, rheumatoid arthritis, atherosclerosis, cardiovascular disease, inflammatory bowel disease, asthma and liver diseases [8, 9, 21–31]. Although some studies suggest that OPN participates in the pathogenesis of SLE, the association of the OPN gene polymorphism and susceptibility and clinical manifestations of the disease are not fully elucidated.

The aim of the study was to investigate the frequency of 707 C/T (rs1126616) polymorphic variants of the OPN gene in Polish patients and healthy controls and its possible association with SLE clinical and laboratory manifestations. This polymorphism is a coding synonymous and exonic splicing enhancer, located on chromosome 89122877 [24]. 707 C/T polymorphism was selected to examine potential associations with SLE because it is located in the exonic region of the OPN gene and has a minor allele frequency of > 5% in the population of European ancestry.

Aim

To our knowledge, this is the first study to investigate the association of 707C/T variant of the OPN gene and the SLE risk in the Polish population as well as its correlation with clinical features of the disease.

Material and methods

Subjects

The study involved 83 Polish patients with SLE (74 women, 9 men) treated at the Department of Dermatology and Venereology, Medical University of Lodz, Poland. Their age ranged from 27 to 73 years (mean: 47.34 ±12.51 years). All patients fulfilled at least four out of eleven American College of Rheumatology (ACR) criteria for SLE classification. This group was selected randomly. ACR classification criteria for SLE were documented using laboratory testing and patient history. 100 healthy subjects (83 women, 17 men) served as controls. Their age ranged from 23 to 71 years (mean: 42.93 ±13.47 years). They did not meet criteria for SLE or any other autoimmune diseases. The study was approved by the Local Ethics Committee (no. RNN/67/08/KE) and all subjects provided written informed consent. The procedures followed were in accordance with the Helsinki Declaration of 1975, as revised in 2000. Short characteristics of SLE patients and control subjects are presented in Table 1.

Table 1. Characteristics of SLE patients and controls. Data are expressed as minimum – maximum, means ± standard deviation or *n*

Parameter	SLE	Controls
Subjects	83	100
Gender (female/male)	74/9	83/17
Age [years]	27–73 (47.34 ±12.51)	23–71 (42.93 ±13.47)
Duration of disease [years]	1–41 (8.49 ±6.56)	
Malar rash	38 (45.8)	
Discoid rash	8 (9.6)	
Photosensitivity	27 (32.5)	
Oral ulcers	12 (14.5)	
Arthritis	67 (80.7)	
Serositis	5 (6.0)	
Renal disorder	6 (7.2)	
Neuropsychiatric disorder	12 (14.5)	
Haemolytic anaemia	14 (16.9)	
Leukopenia	39 (47.0)	
Lymphopenia	13 (15.7)	
Thrombocytopenia	23 (27.7)	
Anti-nucleosome Ab	9 (10.8)	
Anti-dsDNA Ab	17 (20.5)	
Anti-Smith Ab	12 (14.5)	
Anti-Ro/SSA Ab	34 (41.0)	
Anti-La/SSB Ab	30 (36.1)	
Anti-RNP Ab	12 (14.5)	
Anti-His Ab	21 (25.3)	
ANA Ab	61 (73.5)	

SLE – systemic lupus erythematosus, Ab – antibodies, dsDNA – double stranded DNA, RNP – ribonucleoprotein, His – histones, ANA – anti-nuclear antibodies.

Selection of OPN polymorphism

The OPN gene is located on chromosomal region 4q21-q25 and is ~7.8kb in length with 7 exons. Selection of the polymorphism in the OPN gene for genotyping in this study was done by using the HapMap database (http://www.hapmap.org/). OPN 707 C/T polymorphism, located in the exonic region of the OPN gene, has a mutant (minor) allele frequency of > 5% in the population of European ancestry.

DNA isolation and genotyping

Genomic DNA was extracted from whole frozen blood using "Blood Mini" kit (A&A Biotechnology, Gdynia, Poland) and following the manufacturer's protocol. DNA concentration was determined using the Qubit® 2.0 fluorometer. 707 C/T genotyping was performed by real-time polymerase chain reaction (RT-PCR, LightCycler, Roche) with the TaqMan® SNP Genotyping Assay (Applied Biosystems, Foster City, California, USA) according to the protocols provided by the manufacturer. Amplification

steps were 95°C for 10 min, followed by 50 cycles at 92°C for 15 s and 60°C for 1 min. The major sequence contains a C allele while the minor variant is a T allele.

Statistical analysis

Statistical analyses were performed using Statistica 12.0 (StatSoft Inc.). To compare the frequency of genotypes and alleles of OPN 707 C/T in SLE patients and the control group, the χ^2 test with Yate's correction or Fisher's exact test was used. Hardy-Weinberg equilibrium (HWE) was determined by Pearson's goodness-of-fit test. The association of each allele with the clinical features of SLE was assessed by the odds ratio (OR) and its 95% confidence interval (95% CI). Differences were considered statistically significant at p-value < 0.05.

Results

The distribution of OPN 707 C/T genotypes was determined according to Hardy-Weinberg equilibrium for patients ($\chi^2 = 0.37$, $p = 0.54$, d$f = 1$) but not for controls ($\chi^2 = 6.25$, $p = 0.01$, d$f = 1$). The deviation from HWE in control samples may be due to the small sample size or the population stratification. Bearing in mind that deviation from HWE in controls could be problematic in association studies, we have compared the distribution of genotypes in the similar case – the control study [38]. They did not differ significantly from our results. Moreover, the minor allele frequency (MAF) in the present study ($T = 0.20$) was similar to GO-ESP MAF ($T = 0.24$) and TOPMED MAF ($T = 0.27$). Therefore, we believe that deviation from HWE in the control group did not affect the results of the study.

The mutant (CT + TT) combined genotypes were observed more frequently than the wild-type (CC) genotype in SLE patients compared to controls ($p = 0.037$, OR = 1.98, 95% CI: 1.08–3.61). However, no significant difference in allelic distribution was found between the analysed groups ($p = 0.139$, OR = 2.19, 95% CI: 0.91–1.55). 707 C/T genotype and allelic frequencies for SLE patients and controls are presented in Table 2.

Clinical manifestations of SLE according to frequency and their association with 707 C/T variants are shown in Table 3. In SLE subjects, no significant association of genotypes with clinical or laboratory parameters was observed ($p > 0.05$).

Discussion

The present study was conducted to investigate the frequency of 707 C/T (rs1126616) polymorphic variants of the OPN gene in Polish patients and healthy controls and its possible association with SLE clinical and laboratory manifestations.

OPN exerts several immunomodulatory effects and thus may play a role in the course of diseases in which immune mechanisms are believed to play a significant role. An elevated level of OPN was found in patients with multiple sclerosis [9, 25], rheumatoid arthritis [8, 9, 26], atherosclerosis [21, 27], inflammatory bowel disease [28, 29], asthma and allergies [30] and systemic lupus erythematosus [15, 16, 18]. Moreover, it was demonstrated that increased OPN correlates positively with SLE disease activity index (SLEDAI) [14, 16, 18] and is associated with antibodies to double-stranded DNA (ds-DNA) [17].

OPN expression is influenced by genetic polymorphisms of its promoter [19]. Polymorphic OPN alleles have been implicated in the mouse model of lupus [31, 32].

There are reports suggesting the association of various polymorphic variants of the OPN gene and SLE susceptibility or its clinical manifestations [33–37], but the 707 C/T (rs1126616) polymorphism was rarely examined. In a study of Han *et al.* [37], analysis of two groups (European-American and African-American), showed that the T (minor) allele of rs1126616 is significantly associated with a higher risk of SLE in male patients.

The main finding of the present study is that 707C/T variation in the OPN gene is related to SLE susceptibility in the Polish population. We demonstrated that the frequency of mutant (CT, TT) genotypes was higher in SLE patients than in controls. Nevertheless, no relationship between studied SNP and clinical or laboratory parameters was observed. Similar results were obtained in a study of Forton *et al.* [38]. The group found no association of 707 C/T polymorphism with cutaneous lupus, gastrointestinal lupus and specific laboratory features

Table 2. Genotype and allele frequencies of OPN707 rs1126616 (707 C/T) single nucleotide polymorphism (SNP) in SLE patients and controls

Genotype/allele	Number of SLE patients	Number of control subjects	*P*-value	OR (95% CI)
CC	43 (0.52)	68 (0.68)		
CT	35 (0.42)	24 (0.24)		
TT	5 (0.06)	8 (0.08)	0.037[a]	1.98 (1.08–3.61)
C	121 (0.73)	160 (0.80)	0.137[b]	2.19 (0.91–1.55)
T	45 (0.27)	40 (0.20)		

[a]P-value of CC vs. CT + TT, [b]p-value of C vs. T. SLE – systemic lupus erythematosus, OR – odds ratio, 95% CI – 95% confidence interval of the odds ratio.

Table 3. Association between clinical manifestations and laboratory profiles of SLE and OPN rs1126616 genotypes

Manifestation	Genotype frequency		Association	
	CC (*n* = 43)	CT, TT (*n* = 40)	*P*-value	OR (95% CI)
Malar rash	23 (53.5%)	15 (37.5%)	0.146	1.92 (0.80–4.61)
Discoid rash	4 (9.3%)	4 (10%)	0.884	0.90 (0.21–3.86)
Photosensitivity	11 (25.6%)	16 (40%)	0.164	0.16 (0.20–1.31)
Oral ulcers	8 (18.6%)	4 (10%)	0.272	2.06 (0.57–7.45)
Arthritis	36 (83.7%)	31 (77.5%)	0.474	1.49 (0.50–4.88)
Serositis	3 (7%)	2 (5%)	0.707	1.43 (0.23–9.00)
Renal disorder	3 (7%)	3 (7.5%)	0.952	0.95 (0.18–5.00)
Neuropsychiatric disorder	6 (14%)	6 (15%)	0.892	0.92 (0.27–3.12)
Haemolytic anaemia	5 (11.6%)	9 (22.5%)	0.193	0.45 (0.14–1.49)
Leukopenia	22 (51.2%)	17 (42.5%)	0.430	1.42 (0.60–3.37)
Lymphopenia	5 (11.6%)	8 (20%)	0.299	0.53 (0.16–1.77)
Thrombocytopenia	11 (25.6%)	12 (30%)	0.653	0.80 (0.31–2.10)
Anti-nucleosome Ab	6 (14%)	4 (10%)	0.582	1.46 (0.38–5.61)
Anti-dsDNA Ab	9 (20.9%)	8 (20%)	0.909	0.94 (0.33–2.67)
Anti-Smith Ab	7 (16.3%)	5 (12.5%)	0.626	1.36 (0.40–4.70)
Anti-Ro/SSA Ab	21 (48.8%)	13 (32.5%)	0.133	1.98 (0.81–4.84)
Anti-La/SSB Ab	19 (44.2%)	11 (27.5%)	0.117	2.09 (0.83–5.23)
Anti-RNP Ab	10 (23.3%)	2 (5%)	0.054	5.76 (1.18–28.18)
Anti-His Ab	9 (20.9%)	12 (30%)	0.484	0.62 (0.23–1.68)
ANA	36 (83.7%)	25 (62.5%)	0.052	3.09 (1.10–8.66)

OR – odds ratio, 95% CI – 95% confidence interval of the odds ratio, Ab – antibodies, dsDNA – double stranded DNA, RNP – ribonucleoprotein, His – histones, ANA – anti-nuclear antibodies.

in a small group of SLE patients and controls. However, in contrast to our study, it was demonstrated that the T allele was associated with opportunistic infections and renal insufficiency, but on the other hand it was found to be protective against avascular necrosis. The same polymorphism was genotyped in Persian and Baloch SLE patients and controls [39]. It was demonstrated that the frequency of CT and TT genotypes was higher in SLE patients with lupus nephritis, but there was no association between the polymorphism and SLE susceptibility.

The present study suggests that individuals with a heterozygous or homozygous mutation (707C/T) of the OPN gene have a higher predisposition to SLE, however, the molecular mechanisms by which this polymorphism contributes to the SLE risk remain uncertain. 707C/T is a synonymous variant (Ala250Ala). Such changes can affect transcription, splicing, mRNA transport, and translation [40, 41]. We speculate that the mutant T allele is associated with an increased OPN gene transcriptional activity. OPN enhances the proliferation and differentiation of B lymphocytes and autoantibody production [10–13], plays a significant role in lymphocytes activation [10] and increases the production of type I interferon [12] and thus can be involved in SLE pathogenesis.

Although our study suggests an association between OPN 707 C/T polymorphism and SLE susceptibility, the results need to be replicated on a larger sample. An additional limitation of the present study is that plasma OPN concentration was not measured, which could have added a stronger support to the final conclusions and could have been correlated with the OPN genotype-dependent differences.

Conclusions

Further studies are needed to elucidate the potential role of assessment of the polymorphism of the OPN gene in pathogenesis and course of the disease in larger populations of different ethnic groups of patients with SLE.

Acknowledgments

This work was supported by grant no. DEC-2011/01/D/NZ5/00316 from the National Science Centre, Poland.

Conflict of interest

The authors declare no conflict of interest.

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