



Contribution of Polymorphism in PPAR α Untranslated Region to The Development of Axial Spondyloarthritis

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Received: 25.11.2019

Accepted: 18.04.2020

Published: 25.06.2020

Abstract

The study aimed to contribute to the overall understanding of axial spondyloarthritis by investigating the polymorphism in 5' untranslated region of peroxisome proliferator-activated receptor alpha gene in patients. The study included 194 patients and 197 controls recruited. The DNA obtained from the samples was genotyped by multiplex Polymerase Chain Reaction and then Matrix-Assisted Laser Desorption Ionization- Time of Flight Mass Spectrometry. Data were analyzed by logistic regression. Five polymorphic regions analyzed in this study, three of the sites were associated with disease risk. An allele in the rs1800204 polymorphic region ($p < 0.001$), C allele in the rs4253657 polymorphic region ($p = 0.040$) and C allele in the rs13909022 polymorphic region ($p = 0.005$) were associated with disease risk. The association of disease risk could not be detected with G and A alleles in the rs881740 ($p = 0.456$) and rs115640476 ($p = 0.674$) polymorphic regions, respectively. PPAR α 5' untranslated region polymorphism, which is



shown to be associated with disease risk, is thought to contribute to the elucidation of the molecular mechanism of the disease. In axial spondyloarthritis, studying the effects of genetic changes in PPAR α on other genes with which it interacts will contribute to the full understanding of the molecular mechanism of the disease.

Keywords: Axial spondyloarthritis; Peroxisome proliferator activated receptor alpha; Genetic predisposition; Disease risk.

PPAR α Kodlanmayan Bölge Polimorfizminin Aksiyal Spondiloartrit Gelişimine Katkısı

Öz

Bu çalışma peroksizom çoğaltıcılar tarafından aktive edilen reseptör alfa geninin 5' ucu kodlanmayan bölge polimorfizminin aksiyel spondiloartrit hastalığının gelişimine olan katkısını araştırmayı ve hastalığın gelişim mekanizmasının aydınlatılmasına katkı sunmayı amaçlamaktadır. Çalışma 194 hasta ve 197 kontrol ile gerçekleştirildi. Örneklerden elde edilen DNA multipleks PCR sonrası matris aracılı lazer dezorpsiyon iyonizasyon uçuş zamanı kütle spektrometresi yöntemi kullanılarak genotipleme işlemi yapıldı. Elde edilen veriler lojistik regresyon ile analiz edildi. Bu çalışma ile analiz edilen beş polimorfik bölgenin üçü hastalık riski ile ilişkilendirildi. rs1800204 polimorfik bölgesinde A alelinin ($p < 0.001$), rs4253657 için C alelinin ($p = 0.040$) ve rs13909022 için C alelinin ($p = 0.005$) hastalık riski ile ilişkili olduğu tespit edildi. rs881740 polimorfik bölgesi için G aleli ($p = 0.456$) ve rs115640476 için A alelinin ($p = 0.674$) hastalık riski ile ilişkisi tespit edilemedi. Hastalık riski ile ilişkisi gösterilen PPAR α 5' kodlanmayan bölge polimorfizminin, hastalığın moleküler mekanizmanın aydınlatılmasına katkı sunacağı düşünülmektedir. Poligenik bir hastalık olan ax-SpA'de, PPAR α 'daki genetik değişikliklerin etkileştiği diğer genler üzerinde ortaya çıkaracağı etkilerin çalışılması hastalığın moleküler mekanizmasının tam olarak aydınlatılmasını sağlayacaktır.

Anahtar Kelimeler: Aksiyal spondiloartrit; Peroksizom çoğaltıcılar tarafından aktive edilen reseptör alfa; Genetik yatkınlık; Hastalık riski.

1. Introduction

Axial spondyloarthritis (axSpA), a polygenic and chronic inflammatory disease with an unknown pathophysiology, affects the axial skeleton and spine. The most common form of axSpA is ankylosing spondylitis (AS) [1]. More than 7 million people worldwide are estimated to be affected by AS [2]. Peroxisome proliferator-activated receptors (PPARs), known as transcription

factors, are members of the nuclear receptor superfamily involved in the regulation of various essential biological processes. PPAR-alpha (PPAR α), a type of PPAR, is a transcription factor that is activated by a fatty acid belonging to a nuclear hormone receptor family [3]. Due to its collective functions in different tissues, PPAR α is an important focus of research. Although it is known to play a role in the development of several chronic diseases and in inflammatory processes, their role in the mechanism of disease development has not yet been fully identified. PPARs function as physiological sensors under different stress conditions and are valuable targets for innovative therapies [4]. Single-nucleotide polymorphisms (SNP) that have a high frequency in the human genome are known to provide important data regarding disease susceptibility, risk, etiopathogenesis and prognosis [5-7]. In addition, the gene expression of the sequences expressed as untranslated regions (UTRs) at the 5' and 3' ends of the gene regions on the genome reportedly determine the affinity of the mRNA to the ribosome, post-translational modifications, stability of the protein molecule and the stability of the three-dimensional structure [8].

In this study, we evaluated the contribution of polymorphisms in PPAR α 5'untranslated region to the development of axSpA.

2. Materials and Methods

2.1. Study groups

This study included 194 ax-SpA patients (141 male and 53 female; mean age 38.10 ± 11.04 ; range 18 to 65 years) and 197 controls (99 male and 98 female; mean age 39.61 ± 10.34 ; range 20 to 69 years) recruited from the department of Rheumatology, İnönü University in Malatya, Turkey. The study protocol was approved by the ethics committee of Malatya Clinical Investigations (2016/44). A complete clinical evaluation was performed for all patients. Healthy individuals were selected for control group. Ethical rules were followed in this study and written consent of volunteers was obtained. Data collection form included information such as gender, age, disease duration, familial disease story and several clinical characteristics (Table 1).

2.2. Genomic DNA isolation

DNA isolation from peripheral blood samples was performed using Invisorb Spin Blood Mini Kit™ (Catalog Number: 1031100300). DNA concentration measurement before multiplex PCR was done using Nanodrop™. To ensure successful multiplex PCR, it was ensured that DNA concentrations were at least 20 ng/ μ l for each sample.

Table 1: Clinical and demographic data

Demographic Data	Case (n=194)	Control (n=197)	p-Value Odds Ratio (%95CI)
Age (Mean \pm SD)	38.10 \pm 11.04	39.61 \pm 10.34	-
Sex (Male)	%72.7	%50.25	<0.001 2.634(1.728-4.014)
Disease duration (Year \pm SD)	8.57 \pm 4.72	-	-
Diagnosis delay (Year \pm SD)	2.82 \pm 1.41	-	-
Diagnosis of lumbar hernia before Ax-SpA (%)	45.36	-	-
Familial Ax-SpA disease story	%28.87	-	<0.001 3.266 (1.888-5.649)
Surgical intervention before symptoms occur	%10.82	%7.11	0.381 1.393 (0.664-2.922)
Suffered severe infectious disease	%11.86	%3.05	0.002 4.434(1.740-11.300)

2.3. Genotyping

Polymorphic regions were amplified by multiplex PCR using designed primer pairs. The reaction was performed as 45 cycles with pre-denaturation at 95°C for 3 min, denaturation at 95°C for 30 sec, binding at 56°C for 30 sec, elongation at 72°C for 1 min and final elongation at 72°C for 5 min. The total volume of reaction was 5 μ l with the 2.5 mM of MgCl₂, 2 ng of sample DNA, 1 enzyme unit, 0.1 μ M of primer and 500 μ M of dNTP as the final concentration in PCR. Next, 0.5 ml of Shrimp alkaline phosphatase was added to the samples for dNTP neutralisation after multiplex PCR, and the samples were then incubated at 37°C for 40 min. The samples were further incubated at 85°C for 5 min for enzyme inactivation. Next, 0.041 μ l 1X enzyme, 0.940 μ l elongation primer and 0.2 μ l termination mixture were added to the samples for the identification of the polymorphic base, and PCR was performed as 42 cycles with pre-denaturation at 95°C for 30 sec, denaturation at 95°C for 5 sec, binding at 52°C for 5 sec, elongation at 80°C for 5 sec and final elongation at 75°C for 2 min. After the single-base elongation reaction, 16 μ l of ddH₂O was added to each sample. The samples were resin-coated before MALDI TOF analysis to optimise background noise. The samples were transferred to 384-element SpectroCHIP® II, and genotype analysis was performed by MALDI TOF. Primer pairs and probe information used in the single-base elongation reaction and multiplex PCR are presented in Table 2.

Table 2: Sequences of Multiplex PCR primers and single base extension probes

SNP Numbers	Primer/Probe Info	Sequence
rs1800204	Forward	ACGTTGGATGAAAGCAAGTGCCTGGTTTC
	Reverse	ACGTTGGATGTCATACACCAGCTTGAGTCG
	Extension Probe	CCTCACAGGGCTTCTTTC
rs881740	Forward	ACGTTGGATGTTTCAGGTACAGTAGGAGGAG
	Reverse	ACGTTGGATGAGCCAACTGGACCAGCTTC
	Extension Probe	GGGTGAGGAGCAAGCCCAGG
rs4253657	Forward	ACGTTGGATGAACAGTTCTAGCCAGGCACG
	Reverse	ACGTTGGATGTGCCTCCTAGGATCAAGCAG
	Extension Probe	CTTGGAAGGCTGAGG
rs115640476	Forward	ACGTTGGATGTGTCCCTGGGGTTTGGCGCT
	Reverse	ACGTTGGATGACAACCCGAAGACCCAGAGT
	Extension Probe	CGGGTGTTTGGCGCTGCGCGGA
rs139090922	Forward	ACGTTGGATGACACGGAAAGCCCACTCTG
	Reverse	ACGTTGGATGCCCATTTCTTGCAGGAACTC
	Extension Probe	CCGGCGATCTAGAGA

2.4. Statistical analysis

The association of SNP alleles and genotypes with disease predisposition was analysed by logistic regression test (SPSS 24.0, Chicago). Power analysis was performed by using PS Power and Sample Size Program 3.1.2. Hardy-Weinberg equilibrium was evaluated by χ^2 analysis. Demographic data were given as mean \pm standard deviation and min/max values. “p” value smaller than 0.05 was regarded as statistically significant.

3. Results

Clinical and demographic data of patients with axSpA and healthy subjects were presented in Table 1. Disease diagnosis was delayed by 2.82 years. In this study, 28% of the patients had a family history of similar disease ($p < 0.001$), 45% of the patients had been diagnosed with lumbar disc herniation before being diagnosed with axSpA. Also 10% of patients had undergone surgery before disease symptoms manifested and 11% of patients had an important infectious disease before disease symptoms were observed ($p = 0.002$). Disease prevalence was 2.6-fold higher in males than in females [$p < 0.001$; OR 95% CI: 2.634 (1.728–4.014)] (Table 1).

In this study, allele and genotype distributions obtained from the analysis of polymorphic regions in PPAR α 5'UTR are presented in Table 3. A total of five polymorphic regions were

analyzed. Three of them were associated with disease risk. A allele in the rs1800204 polymorphic region was found to be associated with disease risk ($p < 0.001$) and a 2-fold increase in disease risk was noted [OR 95% CI: 2.262 (1.462–3.499)]; C allele in the rs4253657 polymorphic region was found to be associated with disease risk ($p = 0.040$) and a 1.5-fold increase in disease risk was noted [OR 95% CI: 1.561 (1.020–2.391)] and C allele in the rs13909022 polymorphic region was found to be associated with disease risk and a 1.8-fold increase in disease risk was noted [OR 95% CI: 1.851 (1.200–2.855)]. The relationship G and A alleles in the rs881740 and rs115640476 polymorphic regions, were not found to be associated with disease risk ($p = 0.456$ and $p = 0.674$, respectively).

Table 3: PPAR- α 5' untranslated region genotyping results

SNP	Alleles		Case (n=194)				Control (n=197)				p-value	OR (95%CI)
	1	2	11	12	22	MAF	11	12	22	MAF		
rs1800204	A	G	24	109	61	0.405	22	79	96	0.312	< 0.001	2.262 (1.462 – 3.499)
rs4253657	C	T	24	84	86	0.340	14	78	105	0.269	0.040	1.561 (1.020 – 2.391)
rs881740	G	A	57	85	52	0.513	41	97	59	0.454	0.456	1.198 (0.745 – 1.926)
rs115640476	A	G	27	95	72	0.384	36	77	84	0.378	0.674	1.099 (0.709 – 1.702)
rs13909022	C	G	31	94	69	0.402	29	73	95	0.332	0.005	1,851 (1.200 – 2.855)

4. Discussion

axSpA is an inflammatory disease which caused deformation of axial skeleton and consequent movement restriction and labor loss [9-11]. One of the major problems associated with axSpA is the 2 to 5 year delay in its diagnosis [12, 13]. Our study is the first in literature to associate polymorphism in PPAR α with disease risk and to identify the polymorphism as an alternative genetic target for early diagnosis, elucidation of molecular mechanisms of the disease and disease risk in patients with axSpA. This study showed that prevalence of disease in males was 2.6-fold higher than in females [$p < 0.001$; OR 95% CI: 2.634 (1.728–4.014)]. This result was consistent with the data obtained in a prevalence study conducted by Esther [14]. The mean delay of 2.82 ± 1.41 years in disease diagnosis observed in our study was showed similarity with the data reported in various studies [13, 15]. 11.86% of the patients (Table 1) had sustained serious

infectious diseases before disease symptoms had manifested [$p = 0.002$; OR 95% CI: 4.434 (1.740–11.300)]. This finding supports the previously reported statement that microorganism activity may have a role in disease development [16, 17]. 28.87% of the patients had a family history of axSpA [$p < 0.001$; OR 95% CI: 3.266 (1.888–5.649)] and the results were consistent with the data obtained by Serrano (38.4%) and Wang (36%) regarding the ethnic and community parameters [18, 19].

Although no study has yet elucidated the role of PPAR α in axSpA development, several scientific studies have reported that PPAR α is associated with the development of different diseases [20-22]. Dhaini et al. state that PPAR polymorphism may be related to the risk of developing cancer [23]. Dong et al. reported that PPAR polymorphism may have a role in the development of diseases such as Type 2 diabetes and metabolic syndrome [24]. Similarly, Andrulionytė et al. reported that the rs1800206 polymorphism increased the risk of developing diabetes [25]. In another study, Qian et al. analyzed the effect of PPAR α and other members of the PPAR superfamily on the risk of developing coronary heart disease. In one of the studies, PPAR α intron polymorphism (7G/C polymorphism [CC+GC vs GG; $p = 0.003$; OR 95% CI: 1.42 (1.13–1.78)] and L162V polymorphism [VV+LV vs LL; $p = 0.031$; OR 95% CI: 0.74 (0.56–0.97)]) were found to be associated with the risk of developing coronary heart disease [26]. Fan et al. found that PPAR α polymorphism increases the risk of developing atherosclerosis and cardiovascular disease [27].

The human PPAR α gene is a 93.2-Kb gene located on chromosome 22 at position 22q12-q13.1 encoding a protein of 468 amino acids [28]. The gene contains a total of 8 exons. The 5' UTR, including the polymorphic regions that are the focus of our study, is encoded by exons 1 and 2 and partially by exon 3 [28, 29]. PPAR α has an important role in reducing inflammation. PPAR α activity has an effect on neutrophils and macrophages in both acute and chronic inflammatory diseases. Leukotriene B4 (LTB4), a potent chemotactic inflammatory eicosanoid, is an endogenous PPAR α ligand. Like other PPAR α ligands, it induces the transcription of the genes of β - and PP-oxidation pathways that neutralize and reduce LTB4, to regulate inflammatory response [30]. PPAR α regulates the duration of the inflammatory response by limiting cytokine expression and inducing genes that metabolize LTB4 [30].

5. Conclusions

The data obtained from the present study showed that the rs1800204, rs115640476 and rs139090922 polymorphic regions located in 5' UTR of PPAR α , which are responsible for suppressing inflammatory response, are increased axSpA risk. Evaluation of results suggests that

changes in 5' UTR can downregulate PPAR α expression. This may suppress LTB4-metabolising genes leading to increased LTB4 activity, which ultimately contributes to the prolonged duration of the inflammatory response. The genes that are involved in the inflammatory response and interact with PPAR α may offer significant opportunities to completely understand the disease mechanisms and to determine appropriate treatment strategies.

Acknowledgement

This study was supported financially by Munzur University Health Faculty Research Budget (2017-DT).

Ethical Considerations

Ethical rules were followed in this study and written consent of volunteers was obtained. The study protocol was approved by the ethics committee of Malatya Clinical Investigations (2016/44).

Conflict of Interest

There is no conflict of interest among the authors.

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