

Investigation of *Poly (ADP-Ribose) Polymerase-1* Genetic Variants as a Possible Risk for Allergic Rhinitis

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Recent studies point toward the involvement of poly (ADP-ribose) polymerase-1 (*PARP-1*) in the pathogenesis of allergic airway inflammation, such as asthma and allergic rhinitis (AR). It has been suggested that inhibition of *PARP-1* provides significant protection against systemic or tissue inflammation in animal models. The objective of this study was to investigate whether single-nucleotide polymorphisms of *PARP-1* gene are associated with genetic susceptibility to AR. We studied the effect of promoter variations and Val762Ala polymorphism of the *PARP-1* gene on the risk for developing AR in a case-control association study with 110 RA patients and 130 control subjects in a Turkish population. The polymorphisms of 410C/T, –1672G/A, and Val762Ala in the *PARP-1* gene were analyzed using the polymerase chain reaction–restriction fragment length polymorphism method. Haplotype analysis of these groups was also performed. The results were statistically analyzed by calculating the odds ratio (OR) and their 95% confidence intervals using χ^2 tests. The heterozygote genotype of the promoter polymorphism (–1672) was significantly found to be associated with susceptibility to AR (OR: 0.56) among the tested single-nucleotide polymorphisms. Haplotypes of *PARP-1* –410, –1672, and 762 were not associated with an increased risk for AR. These results raise the possibility that the promoter (–1672) polymorphism of the *PARP-1* gene may be a risk factor for AR.

Introduction

ALLERGIC RHINITIS (AR) is a chronic inflammatory disease characterized by mucosal inflammation in response to allergen exposure. The pathogenesis of AR and similar allergic diseases involves complex interactions of environmental exposure to allergens and respiratory irritants (Shusterman *et al.*, 2003; Wang, 2005; Bousquet, 2008) as well as genetic predisposition of the individual patients (Dávila *et al.*, 2009). Many susceptibility loci for AR and atopy have been described in various Western and Eastern populations (Bauchau and Durham, 2004; Mou *et al.*, 2010; Wang, 2011). Results from several studies have suggested that interactions of multiple genes and environmental factors cause susceptibility to AR. Poly (ADP-ribose) polymerase-1 (*PARP-1*) may be a good candidate gene as deletion experiments of this gene and therapeutic approaches using *PARP-1* inhibitors indicated an improvement in systemic or tissue inflammation. The absence of functional *PARP-1* enzyme in *PARP-1* knockout animals provided significant protection against miscellaneous manifestations of airway inflammation upon allergen exposure

(Boulares *et al.*, 2003; Suzuki *et al.*, 2004; Virág *et al.*, 2004; Dávila *et al.*, 2009). These data suggest the *PARP-1* activity to be a critical step in allergen-induced and airway inflammation. *PARP-1* also activates various proinflammatory pathways by interacting with NF- κ B (Csiszar *et al.*, 2008). However, precise mechanisms underlying the role of the *PARP-1* enzyme in the inflammatory process have not been delineated yet.

PARP-1 is known to be involved in various processes, such as DNA repair, proliferation, transcription, regulation, apoptosis, genomic stability, stress response, and malignancy. It is believed that this enzyme mediates inflammation through promotion of cell death by means of ATP depletion as well as transcription of inflammatory factors (Datta *et al.*, 2011). Upon activation, *PARP-1* catalyzes the formation of poly (ADP-ribose) chains by transferring (ADP-ribose) from NAD onto itself and nuclear acceptor proteins. *PARP-1* activation has been also implicated in the initial inflammatory response by modulating expression of inflammation-related genes such as interleukins.

The *PARP-1* gene is located on chromosome 1q42 and consists of 23 exons spanning 43 kb. Recently, several

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nucleotide variants in the *PARP-1* gene have been reported (Cao *et al.*, 2007; Jin *et al.*, 2010; Liu *et al.*, 2010). Among these polymorphisms, codon 762 variant (exon 17, nucleotide 2444 T to C, and V to A) causes about 40% decrease in the *PARP-1* enzymatic activity through increasing Km (Wang *et al.*, 2007). The promoter of the gene contains a polymorphic dinucleotide CA repeat in the 5'-flanking sequence of the N-terminal binding domain. Interestingly, this multiallelic polymorphism is located close to the binding site of the transcription factor Yin Yang 1, and therefore might effect *PARP-1* transcription. Additionally, four sequence variations have been identified in this region: C410T, Poly(A)n, C13362T, and G1672A (Kato *et al.*, 2000; Pascual *et al.*, 2003). Multiple binding sites for transcription factors have been identified in the *PARP-1* gene promoter region, and thus, genetic variants in this region might effect *PARP-1* expression. Polymorphisms in the promoter region of the gene (−410 and −1672) usually account for gene expression level variability. In the light of this data, *PARP-1* would be an attractive candidate gene for AR, opening the question whether individual variations in *PARP1* expression might account for different susceptibilities to inflammatory diseases. In this study, we aimed to examine the promoter (−410 and −1672) and codon 762 (V/A) polymorphisms of the *PARP-1* gene in AR patients for determining their association with susceptibility to AR.

Materials and Methods

Subjects

One hundred ten consecutive and unrelated patients with AR were included in this study. They consisted of 47 females and 63 males, ranging in age from 17 to 57 years (mean, 28.9 years).

The AR patients were recruited from our outpatient clinic at the Haseki Research and Training Hospital. The diagnosis of AR was based on symptoms of sneezing, watery rhinorrhea, and nasal obstruction, and the positive results of a skin test. The skin test was performed with six common aeroallergens, such as house dust mites, house dust, grass mix, tree pollens, animal dander, and molds (SPT, Allergopharma; Merck-KGaA). Treatment with sodium cromoglycate, antihistamines, and corticosteroids was stopped at least 4 weeks before entering the study. All patients with AR had histories of all salient symptoms, and had a positive skin test for at least 2 years. No subject had experienced an infection of the respiratory tract 4 weeks before the investigation. Subjects who suffered from AR that required the regular use of inhaled corticosteroids or use of systemic corticosteroids were excluded from the study. The control group consisted of 130 healthy subjects with no personal or family history of allergic diseases, cancers, or genetic diseases. There were 57 females and 73 males ranging from 18 to 63 years old (mean, 31.2 years). The control group was chosen from individuals who did not have any nasal complaints or any nasal pathology (nasal septal deviation, hypertrophic concha, and nasal polyp) which might cause nasal congestion. The controls without AR were recruited from the general population, and had no evidence of inflammation in the nasal cavity. In addition, patients had received comprehensive medical testing at the Haseki Research and Training Hospital. All subjects in this study were Turkish and lived in the same area. Informed consent was obtained from all patients and control subjects,

and the study protocols were approved by the Institutional Review Board, Haseki Research and Training Hospital.

PARP-1 polymorphisms genotyping

For *PARP-1* Val762Ala polymorphism, we optimized HRM allele discrimination using the following primers: forward 5'-CCTAAGTCGGGGGCTTCTTTTC-3' and reverse 5'-GTAGGCCACCTCGATGTCCAGC-3'. The polymerase chain reaction (PCR) product length was 80 bp. The polymorphic region at 762 valine coding GTG changed into alanine coding GCG, which was differentiated using the HRM technique. Reactions were performed on a RotorGene 6000 device (Corbett). Reaction volumes comprised 10 μ L 2 $\times$ SYBR[®] Premix Ex Taq[™] (Takara Bio, Inc.), 0.3 μ M of each primer, and 2 μ L of DNA (~100 ng) in a final volume of 20 μ L. The temperature cycling parameters were 95°C for 1 min; 35 cycles of 95°C for 10 s, 60°C for 15 s; followed by 72°C for 30 s. Amplification was followed by HRM (75–90°C) increasing by 0.1°C at each step. Each sample PCR was duplicated. The results for the real-time PCR experiments were analyzed using the RotorGene 6000 software v 1.7 (Corbett). Two known polymorphic alleles carrying a DNA sample were included in each run for calibration and accurate assignment of HRM alleles.

Determination of the C410T and G1672A polymorphisms was PCR-restriction fragment length polymorphism with standard amplification protocols by using appropriate restriction endonucleases (*Dde* I for C410T and *Bst* UI for G1672A) (Kato *et al.*, 2000). Primer sets were as follows: C410T 5'-TCCAGTGGCAACTATCAT-3' (forward), 5'-GTT GTGAGACATAGGCCGAATC-3' (reverse); G1672A 5'-GCG AGACCGCGAGACCCTGTCCCTAA-3' (forward), 5'-TCCC CCTTTTATTTTGAGACTG-3' (reverse).

Data analysis

Hardy–Weinberg equilibrium analyses were performed with the Hardy–Weinberg equilibrium calculator (www.oege.org/software/hwe-mr-calc.shtml) (Rodriguez *et al.*, 2009). The χ^2 test was performed to compare genotype and allele frequencies between patients and healthy controls with the GraphPad Prism software (GraphPad Software). Haplotype frequencies were estimated using the Haploview software v.4.0.

Results

The distribution of the *PARP-1* (−410), (−1672), and (762) genotypes in control groups were within the range of Hardy–Weinberg equilibrium. The distribution of the genotype and allele frequencies of all polymorphisms studied is shown in Table 1. When compared to homozygotes for the *PARP-1* (−1672) GG genotype, a significant increase in risk for AR was found in (−1672) GA heterozygotes (odds ratio [OR]: 0.56, 95% confidence intervals [95% CI]: 0.32–0.98, 0.0396), conferring a 1.78-fold increase in AR risk; however, no such significant difference between groups was observed for neither of the genotypes of *PARP-1* (−410) and (762) polymorphisms. In addition, there were no significant differences between the AR and control groups ($p > 0.05$) in the allele frequencies of *PARP-1* (−410), (−1672), and (762) polymorphisms.

We also investigated if these three polymorphisms were in linkage disequilibrium and if there were any common

TABLE 1. DISTRIBUTION OF 762 (–410) AND (–1672) POLYMORPHISMS OF *PARP-1* IN ALLERGIC RHINITIS PATIENTS AND CONTROLS

Genotype/allele	Cases n (%)	Controls n (%)	OR (95% CI)	p-Value
<i>PARP-1</i> (762)	103	128		
VV	45 (43)	61 (47)	1 (Reference)	
VA	44 (42)	52 (40)	0.87 (0.49–1.52)	0.628
AA	14 (13)	15 (11)	1.28 (0.85–1.92)	0.224
T (Val) allele frequency	134	119	Reference	
C (Ala) allele frequency	72	82	1.282 (0.8583–1.916)	0.260
<i>PARP-1</i> (–410)	109	124		
CC	44 (40)	50 (40)	1 (Reference)	
CT	54 (49)	59 (47)	0.96158 (0.5557–1.664)	1.000
TT	11 (10)	15 (12)	1.200 (0.4990–2.886)	0.825
C allele frequency	142	159		
T allele frequency	76	89	1.046 (0.7147–1.531)	0.846
<i>PARP-1</i> (–1672)	102	128		
GG	33 (32)	57 (44)		
GA	64 (62)	62 (48)	0.5609 (0.3226–0.9751)	0.039
AA	5 (5)	9 (7)	1.042 (0.3220–3.373)	0.945
G allele frequency	130	176		
A allele frequency	74	80	0.7985 (0.5411–1.178)	0.256

The *p* indicates *p*-value of χ^2 test.

All three single-nucleotide polymorphisms (SNPs) were not always analyzed in each sample.

CI, confidence interval; OR, odds ratio; *PARP-1*, poly (ADP-ribose) polymerase-1.

haplotypes associated with disease, and rare haplotypes (with frequency <5%) were excluded in the association analyses. The most common haplotypes of the three polymorphisms, calculated by Haploview software, are summarized in Table 2. However, none of the haplotypes were associated with overall risk for rhinitis.

Discussion

Although AR is an inflammatory disease of unknown cause, several investigators have suggested that there may be various genetic risk factors affecting susceptibility to this disease (Shusterman *et al.*, 2003; Bousquet, 2008). However, the details of underlying mechanisms have remained unclear. *PARP-1* may be a piece in the AR puzzle, because it may modulate the transcription of inflammation-linked genes (Csiszar *et al.*, 2008). *PARP-1* has been proposed to regulate NF- κ B-dependent transcription and synthesis of inflammation mediators. In addition, the relationship between *PARP-1* activity and inflammation has been widely investigated and the link with inflammation was firmly established by the pharmacological inhibition of PARP activity, which allowed the attenuation of the inflammation response (Giansanti *et al.*,

2010). Therefore, the gene for *PARP-1* might be a good candidate based on the observations made by knockout mice and promising therapeutic approaches to inflammatory diseases by using *PARP-1* inhibitors.

Several nucleotide variants in the *PARP-1* gene have been reported (Cao *et al.*, 2007; Jin *et al.*, 2010; Liu *et al.*, 2010). Among these, the codon 762 variant (exon 1, 2444 T to C, V to A) is present in about 5–33% of the general population and causing about a 40% decrease in the *PARP-1* activity. Wang *et al.* (2007) have shown that the *PARP-1* Val762Ala polymorphism reduced the enzymatic activity of *PARP-1* through increasing *K_m*. We previously reported that the Val allele for codon 762 variant of the *PARP-1* gene is associated with a risk for asthma (Tezcan *et al.*, 2009). In this article, we suggested that in this single-nucleotide polymorphism, which leads to valine to alanine exchange at position 762 of the *PARP* gene, the alanine allele may have a protective effect for asthma. Taking into account both the findings and the information that both asthma and AR are caused by an allergic reaction and the two conditions frequently overlap, and that the Val allele might also be present at higher frequencies in patients with AR and that Val762Ala polymorphism might be a risk factor for developing AR in people. We did not find any publicly released data regarding this association of *PARP-1* Val762Ala polymorphism with AR. However, the results of the present study show that the polymorphic marker Val762Ala located in the coding region of *PARP-1* is not associated with AR development in the Turkish population. This result was in accord with the earlier report by us in rheumatoid arthritis, which is another inflammatory disease. A complex structure due to different diseases needs to be taken into consideration when interpreting these results. Thus, there is no doubt that further studies need to be undertaken to dissect the exact role of this polymorphism on inflammatory diseases.

On the other hand, there are studies that indicate the potential association of variations in the regulatory region of

TABLE 2. HAPLOTYPE FREQUENCIES OF *PARP-1* GENE POLYMORPHISMS IN PATIENT GROUP WITH RHINITIS AND CONTROL GROUP

<i>PARP-1</i> haplotypes (SNP)			Haplotype frequency		
762	–410	–1672	% Cases	% Controls	p-Value
T	G	T	23.7	37.2	0.057
C	G	T	16.9	16.4	0.934
T	A	T	19.5	12.1	0.198
T	G	C	7.3	16.9	0.057
T	A	C	11.1	9.4	0.719
C	G	C	10.2	6.3	0.377

PARP-1 gene with inflammation-related diseases such as Parkinson's disease and Alzheimer's disease (Infante *et al.*, 2007a, 2007b), and showed that *PARP-1* –410 and 1672 haplotypes were associated with an increased risk for Alzheimer's disease. *PARP-1* promoter haplotypes have also been related to susceptibility to chronic inflammatory diseases like rheumatoid arthritis. Although functional significance of *PARP-1* (–1672G/A), which is located close to the binding of the transcription factor Yin Yang 1 and *PARP1* (–410C/T) promoter polymorphisms, is still not known, it has been speculated that variation at these loci may play major roles in the inflammatory process by affecting the proinflammatory cytokines involved in the pathogenesis of various inflammatory diseases caused by overexpression of the *PARP-1* gene. Interestingly, the *PARP-1* –1672G/A polymorphism is located close to the binding of the transcription factor Yin Yang 1. As far as we know, there is no published study exploring the associations between the mentioned variations of *PARP-1* polymorphisms and AR. Therefore, we also attempted to elucidate the significance of these polymorphisms in AR patients.

A total of 110 AR patients and 130 healthy control subjects were enrolled in a case-control study to compare the incidence of the promoter (–410 and –1672) and codon 762 (V/A) polymorphisms of the *PARP-1* gene in these two populations. The AR patients and control subjects were well matched for age, gender, and ethnic origin. In our study, although the distribution of the *PARP-1* (–410) genotypes did not show significant differences between cases and controls, more adult subjects with AR than controls were heterozygous for *PARP* (–1672G/A) polymorphism. In this study, we found a 1.78-fold greater incidence of the GA genotype in AR patients compared with the healthy control population. This result indicates that heterozygosity at these loci was associated with susceptibility to AR. In fact, molecular heterosis may be present in 50% of all gene associations (Comings and MacMurray, 2000), including variations in the *PARP-1* gene (Infante *et al.*, 2007b; Tezcan *et al.*, 2009). Whether to constitute or to reject an association of the *PARP* (–1672G/A) polymorphism, its relevance to AR should be extensively evaluated in several well-characterized populations. At present, although information is lacking about the expression levels of *PARP-1* in the tissue and cells associated with disease activity in the upper and lower airways of patients with AR who have different *PARP-1* (–1672) genotypes, downregulation of *PARP-1* may be protective for disease.

In summary, the current studies raise the possibility that the promoter (–1672) polymorphism of the *PARP-1* gene may be a risk factor for AR. Additional studies with larger numbers of patients are required to clarify the role of this polymorphism and other nucleotide polymorphisms tested as a risk factor in AR.

Author Disclosure Statement

None of the authors have any conflicts of interest to declare.

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