Association between p53 Codon 72 (Arg72Pro) Polymorphism and Primary Open-Angle Glaucoma in Iranian Patients

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ABSTRACT

Background: Glaucomatous neuropathy is a type of cell death due to apoptosis. The p53 gene is one of the regulatory genes of apoptosis. Recently, the association between the p53 gene encoding for proline at codon 72 and primary open-angle glaucoma (POAG) has been studied in some ethnic groups. This study is the first association analysis of POAG and p53 codon 72 polymorphism in Iranian patients.

Methods: A cohort of 65 unrelated patients with POAG (age range from 12-62 years, mean ± SD of 40.16 ± 17.51 years) and 65 unrelated control subjects (without glaucoma, age range of 14-63 years, mean ± SD of 35.64 ± 13.61 years) were selected. In Iranian POAG patients and normal healthy controls, the p53 codon 72 polymorphism in exon 4 was amplified using polymerase chain reaction. The amplified DNA fragments were digested with the BstUI restriction enzyme, and the digestion patterns were used to identify the alleles for the polymorphic site.

Results: Comparisons revealed significant differences in allele and genotype frequencies of Pro72Arg between POAG patients and control group. A higher risk of POAG was associated with allele Pro (OR = 2.1, 95% CI = 1.2–3.4) and genotype Pro/Pro (OR = 3.9, 95% CI = 0.13-12.7).

Conclusion: The p53 Pro72 allele was more frequent in Iranian POAG patients than in the control group (P<0.05). The present findings show that the individuals with the Pro/Pro genotype may be more likely to develop POAG. However, additional studies are necessary to confirm this association.

Received 29 January 2014; revised 7 July 2014; accepted 16 July 2014

Keywords: Primary open-angle glaucoma (POAG), Glaucoma, p53, Codon 72, Iran

INTRODUCTION

Glaucoma is currently the main cause of irreversible, chronic, degenerative optic neuropathy, which affects approximately 70-80 million people worldwide [1, 2]. This disease is the second leading cause of vision loss, and the number of people suffer from this disease is expected to increase due to the aging [3]. Indeed, glaucoma comprises a group of neurodegenerative disorders that involve apoptotic death of retinal ganglion cells. Glaucoma can be roughly divided into three main types: open-angle, closed-angle, and developmental [4]. Primary open-angle glaucoma (POAG) is the most common type of glaucoma characterized by a complex inheritance, slow, and irreversible apoptotic death of retinal ganglion cells, a unique optic nerve neuropathy resulting in loss of vision, adult onset, a gonioscopically open-angle, and a reduced outflow facility that originates elevated intraocular pressure [3, 5, 6].

It is known that POAG is a multifactorial disease in which genetic and environmental factors are involved [7]. Various risk factors have identified for development of open-angle glaucoma, including age, elevated intraocular pressure, exfoliation syndrome, race, myopia, diabetes, and decreased perfusion pressure [8, 9]. However, a positive family history remains among the most important ones established for POAG. Genetic studies have identified that specific genes (such as MYOC, ASB10, WDR36, NTF4, and TBKI) contribute to the pathogenesis of POAG [10-12].

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The common polymorphism of \( p53 \) at codon 72, either encoding proline or arginine, has been associated with a genetic factor associated with clinical outcome in several different studies. For the purposes of this study, the narrow definition of affected POAG status was based on open angles, glaucomatous optic neuropathy, and visual field defects consistent with glaucoma. Glaucomatous optic neuropathy was defined as a narrowed neuroretinal rim, notching of the neuroretinal rim, and/or marked asymmetry in the cup-to-disc ratio. Glaucomatous visual field defects were based on the Glaucoma Hemifield Test and clinician interpretation. The diagnosis criteria used for patient recruitment were used to increase in intraocular pressure and glaucomatous damage to the optic nerve head, and/or glaucomatous damage of the visual field. According to the exfoliative glaucoma patients, the additional criterion of presence of pseudo exfoliative material on e.g. the iris or lens was required for diagnosis. Exclusion criteria included eye surgery and use of glaucoma eye drops for more than two weeks. All information was devoid of identifiers and kept in a database. The data collection was in accord with an approval by the Shahid Sadoughi Medical Science University (Yazd, Iran).

The \( p53 \) codon 72 polymorphism genotyping. Venous blood (20 ml) was extracted from all participants and distributed into 4 ml EDTA tubes and kept in a database. The data collection was disclosed. The study was performed using statistical software package SPSS 17.0 software. The Hardy-Weinberg equilibrium was performed using statistical software package SPSS 17.0 software. The Hardy-Weinberg equilibrium was performed using statistical software package SPSS 17.0 software. The Hardy-Weinberg equilibrium was performed using statistical software package SPSS 17.0 software. The Hardy-Weinberg equilibrium was performed using statistical software package SPSS 17.0 software.
estimated separately for patients and controls. For statistical analysis, the distribution of this polymorphism in the control and POAG groups was compared using the chi-square test. The association between p53 codon 72 polymorphism and POAG was assessed by computing the odds ratio (OR) and 95% confidence intervals (95% CI). The results were considered statistically significant when the probability of findings occurring by chance was less than 0.05 (P<0.05).

RESULTS

The frequencies of the genotypes in the control group and the POAG patients are shown in Table 1. The proline allele is identified by the presence of a single fragment of 312 bp, and the arginine allele by two fragments of 259 and 53 bp, respectively. Heterozygous samples displayed all three fragments (Fig. 1). Using the chi-square test, the distribution of the p53 codon 72 polymorphism was compared, and a significant difference was found between groups POAG patients and controls (P<0.008). The distribution of the genotypes in the POAG group revealed 17 (26.1%) Arg homozygotes, 27 (41.6%) Arg/Pro heterozygotes, and 21 (32.3%) Pro homozygotes. The distribution of the genotypes in the control group revealed 25 (38.5%) Arg homozygotes, 32 (49.2%) Arg/Pro heterozygotes, and 8 (12.3%) Pro homozygotes. The allelic frequencies in the POAG group were 61 (0.47%) Arg and 69 (0.53%) Pro, while in the control group were 82 (0.63%) Arg and 48 (0.37%) Pro. The frequency of the Pro allele was significantly higher in the POAG group. In this study, there was a significant association between the Pro allele of p53 Arg72Pro and POAG in Iranian patients. A higher risk of POAG was associated with allele Pro (OR = 2.1, CI 1.2–3.4) and genotype Pro/Pro (OR = 3.9, CI 0.13-12.7).

DISCUSSION

The ultimate goal for glaucoma genetic research is to identify the specific set of gene mutations that confer high-risk of developing glaucoma, which could then be

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Table 1. Distribution of p53 Arg72Pro polymorphism in POAG patients and controls

<table>
<thead>
<tr>
<th>p53 Arg72Pro</th>
<th>POAG (%)</th>
<th>Controls (%)</th>
<th>OR* (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotypes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg/Arg</td>
<td>17(26.1%)</td>
<td>25(38.5%)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Arg/Pro</td>
<td>27(41.6%)</td>
<td>32(49.2%)</td>
<td>2.2 (0.89-5.1)</td>
<td>0.008</td>
</tr>
<tr>
<td>Pro/Pro</td>
<td>21(32.3%)</td>
<td>8(12.3%)</td>
<td>3.9 (0.13-12.7)*</td>
<td></td>
</tr>
</tbody>
</table>

Allele

<table>
<thead>
<tr>
<th></th>
<th>POAG (%)</th>
<th>Controls (%)</th>
<th>OR* (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arg</td>
<td>61(0.47%)</td>
<td>82(0.63%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Pro</td>
<td>69(0.53%)</td>
<td>48(0.37%)</td>
<td>2.1 (1.2–3.4)*</td>
</tr>
</tbody>
</table>

POAG, primary open-angle glaucoma; OR*, odds ratio; 95%CI, 95% confidence intervals (*P<0.05).

Fig. 1. Genotype analysis by digestion of RFLP-PCR products. Lane 1 is Pro homozygote sample, lane 2 is Arg/Pro heterozygote, and lane 3 is homozygote samples for Arg allele. Lane M, 100 bp DNA ladder (Fermentas).
used to screen patients before clinical phenotypes manifestation. It is suggested that POAG is most likely caused by the interactions of multiple genes and environmental factors. It appears that most of the association studies for POAG have investigated only single genes or single gene alleles without accounting for contributions from gene-gene and gene-environment interactions [16].

The pathogenesis of POAG is genetically heterogeneous and complex. Although, it has been shown that several genetic loci and genes have associated with POAG, the major genes that confer significant susceptibility remain unknown [7]. Interestingly, p53 has been involved within the development of POAG [7]. In recent decade, p53 as a main candidate susceptibility gene to the glaucoma has studied broadly [17]. There have been inconsistent reports regarding the increased risk of glaucoma and genetic variations within p53 [14]. Originally, an association was detected between POAG and SNP in exon 4 of p53 at codon 72 in a Chinese population [14]. However, two other studies conducted in Australia and India did not report such association in POAG patients [17, 18].

The p53 ability to trigger apoptosis depends on the residue occupying position 72 in the polypeptide chain. The p53 isoform with Arg72 more efficiently induces apoptosis, and its content in the mitochondrial fraction is almost one order of magnitude higher than the content of p53 with Pro72 [19]. In addition, the Arg72 isoform of p53 more efficiently activates and interacts with p53 as compared to the Pro72 isoform. In contrast, the Pro72 allele appears to induce a higher level of G1 arrest than the Arg72 allele. Therefore, it seems that the Arg72 and Pro72 alleles are functionally distinct, and these differences may influence the risk of cancer development [20]. Dumont et al. [21] reported that the Arg72 allele had up to 15 fold increased apoptotic ability compared with the Pro72 allele in both inducible cell lines and cells with endogenous p53 homozygous for each allele. Storey et al. [22] found that when p53 was degraded by human papilloma virus, the Pro form was seven times longer than the Arg form. Therefore, the hypothesis is that the Arg form of p53 in residue 72 may be responsible for the less potent effects when the cell must be replicated. Therefore, the results of this study indicated that the Pro form allele is a significant risk factor for POAG [22].

Epidemiological studies have reported that the Arg72 allele is more common in Northern Europeans than in Africans or African-Americans [23, 24]. The Pro/Pro genotype is found in 47% of sub-Saharan Africans and 28% of Japanese and 8% of European Whites, a population with an increased prevalence of normal tension glaucoma [12]. It is hypothesized that p53 codon 72 alleles were latitude dependent. Interestingly, it has been shown that this latitude dependency is tightly associated with winter temperature [23, 24]. Furthermore, it seems that p53 codon 72 polymorphism may be involved in the pathogenesis of POAG in Asians, but not in Caucasians [24]. These findings display the potential role of ethnic difference in genetic background and the environment where they live in.

This investigation is the first association study between the POAG and p53 gene in the Iranian population. The results have shown that the distribution of the p53 gene codon 72 polymorphism in Iranian POAG patients and the healthy control group is significantly different (P = 0.007) (Table 1), confirming the idea of the relation between apoptosis and neuropathy. As mentioned previously the association of the codon 72 polymorphism in p53 gene has not been compatible in most studies. In a meta-analysis study by Guo et al. [25], it has been shown that p53 codon 72 (Pro/Pro vs. Arg/Pro + Pro/Pro) and intron 316-bp insertion (Ins vs. Del) polymorphisms were associated with increased risk for POAG. However, no significant association was identified between rs1042522 and POAG in studies carried out in Indian, Australian, Japanese, Turkish, and Brazilian populations [16]. It is possible that the polymorphism is associated with the Iranian POAG patients, but not with other ethnic groups [18]. The results showed that the Pro allele was prevalent in Iranian POAG patients (OR = 2.389, 95% CI: 1.14 to 5.01) (Table 1). In contrast, Daugherty CL et al. [7] have found that the Arg allele is associated with increased risk of POAG. Some reports have revealed that the Pro allele homozygote is a risk factor for other conditions such as lung and hepatocellular carcinoma [7, 22]. Chen et al. [26] noted a significant association between Pro homozygotes and invasive bladder cancer in Chinese people. Furthermore, researchers have found that lung carcinoma patients with either p53 Arg or Pro homozygotes have worse prognoses when compared with patients with the heterozygous form [26].

Population studies of the association between the p53 Pro72Arg polymorphism with nerve diseases have yielded rather discrepant data. It has been reported that Pro and Arg alleles have association with neuropathy in POAG and early onset Leber’s hereditary optic neuropathy patients, respectively [26]. Moreover, the association between Pro72Arg with glaucoma has not been confirmed in different works [24]. This diversity may be due to racial variation. The studies mentioned above might suggest that the dominant p53 Pro form is a risk factor for disease in the Chinese population. Nevertheless, it is suspected that the Pro form of p53 gene codon 72 induces the instability of ocular ganglion cells, and the Pro form allele fails to protect ganglion cells from apoptosis. The Arg allele was
observed to be related to cancer. It is suggested that the proteomics studies may find the exact effect of Pro on POAG [14].

In summary, the p53 Arg72 allele was more frequent in the Iranian POAG patients compared with those in the control group (P<0.05). The result of this study suggests that patients with the Pro/Pro genotype may be more likely to develop POAG; however, additional studies are necessary to confirm this association.

ACKNOWLEDGMENTS

This research was supported, in part, by a grant from Shahid Sadoughi University of Medical Sciences, Yazd, Iran. The authors would like to thank Professor Seyed Mahdi Kalantar and Professor Mohammad Hassan Sheikhha for providing the opportunity and impetus to perform this study.

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