Primary open angle glaucoma in a Caucasian population is associated with the p53 codon 72 polymorphism

Christopher L. Daugherty,1 Hilda Curtis,1 Tony Realini,1 Judie F. Charlton,1 Sepideh Zareparsi1,2

1Department of Ophthalmology, West Virginia University, Morgantown, WV; 2Department of Biochemistry, West Virginia University, Morgantown, WV

Purpose: Apoptosis has been implicated as the mechanism for retinal ganglion cell death in primary open-angle glaucoma (POAG), a complex neurodegenerative disease. There have been inconsistent reports regarding increased risk of POAG and a polymorphism (Arg72Pro) within the tumor suppressor gene, p53. The goal of this study was to examine the role of this polymorphism in susceptibility to POAG in a Caucasian population from the United States.

Methods: We generated genotypes in 191 unrelated Caucasian POAG patients and 167 unrelated Caucasian controls for the following polymorphisms within p53: rs1042522 (Arg72Pro), rs17878362 (16 bp Ins/Del), and rs1800371 (Pro47Ser) by PCR amplification followed by restriction digestion and sequence analysis.

Results: There was a significant difference in genotypic frequencies for rs1042522 (Arg72Pro) between POAG patients and controls ($\chi^2=9.56$, $p=0.008$). Individuals who were homozygous for the arginine allele have a 1.9-fold significantly increased risk of developing glaucoma (95%CI: 1.16-2.82, $p=0.01$). Interestingly, we found that the frequency of the arginine allele was even higher in the normal-tension glaucoma (NTG) subtype compared to high-tension POAG (0.81 versus 0.76).

Conclusions: Our preliminary results indicate that the arginine variant of rs1042522 within p53 is associated with increased risk of POAG. This variant has increased apoptotic potential, thus the retinal ganglion cells in carriers of the arginine allele may have greater susceptibility to apoptosis.

Glaucoma, the second leading cause of blindness in the world, includes a group of eye disorders characterized by visual field defects, retinal ganglion cell death, and progressive degeneration of the optic nerve. Adult-onset primary open-angle glaucoma (POAG) represents the most prevalent form of glaucoma, and affects 33 million individuals worldwide [1]. It is recognized that POAG is a multi-factorial disorder involving the role of multiple genes as well as environmental factors. The major risk factors for the development of POAG include advanced age, race, elevated intraocular pressure (IOP), and family history. Genome-wide linkage analysis in families with an autosomal dominant form of POAG have resulted in identification of eight chromosomal regions that harbor genes involved in POAG [2-9]. To date, mutations in three genes (myocilin [MYOC], optineurin [OPTN], and WD repeat domain 36 [WDR36]) have been found to be associated with POAG [8,10,11]. However, these mutations only account for 3-5% of all POAG cases [12]. Thus, the genetic basis of the majority of POAG cases remains unknown.

The death of retinal ganglion cells in glaucoma has been the subject of extensive research, which has implicated apoptosis as the pathway for programmed cell death (reviewed in [13]). One of the important regulatory proteins in apoptosis is the tumor suppressor protein p53, known as the “guardian of the genome”. p53 can promote apoptosis through a transcription-dependent mechanism or independent of transcriptional regulation. Under normal conditions, p53 levels and activity are tightly regulated. Upon diverse forms of cellular stress the steady state levels and transcriptional activity of p53 are considerably increased. Moreover, it has been shown that p53 is active in retinal ganglion cells. Specifically, elevated levels of p53 have been detected within the inner retina after transient retinal ischemia [14]. In heterozygote mice with a p53 null mutation, there was significant resistance to ischemia and preservation of the inner retinal layer [14].

Interestingly, p53 has been implicated in development of POAG. There have been inconsistent reports regarding increased risk of glaucoma and genetic variations within p53. An association was originally detected between POAG and a SNP in exon 4 of p53 at codon 72 (rs1042522-Arg72Pro) in a Chinese population [15]. However, two additional groups did not observe this association in a group of Indian and Tasmanian POAG patients [16,17]. Examination of a 16 base pair (bp) insertion/deletion polymorphism located within p53 intron 3 (rs17878362) has provided suggestive evidence that a specific haplotype based on this variation and the p53 exon 4 SNP is associated with POAG [16,18]. However, it should be noted that each of these studies examined different...
This study was reviewed and approved by the West Virginia University Institutional Review Board. All subjects provided written informed consent and authorization to use protected health information. For this retrospective case-control association study, subjects with primary open-angle glaucoma and subjects without glaucoma were recruited from the West Virginia University Eye Institute that have the unique feature of representing the Appalachian population. In our cohort, there is evidence for an association between POAG risk and the \( p53 \) Arg72Pro polymorphism based on the results provided below.

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### RESULTS

Our cohort consisted of 167 unrelated Caucasian controls and 191 unrelated Caucasian POAG patients, of whom 52 had NTG. Subject characteristics are summarized in Table 2. The majority of subjects were of European descent and had the unique feature of representing the Appalachian population.

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### TABLE 1. PRIMER SEQUENCE AND RESTRICTION ENZYMES USED FOR GENOTYPING THE POLYMORPHISMS WITHIN \( p53 \).  

| SNP     | Distance | Enzyme | F-primer       | R-primer       |
|---------|----------|--------|----------------|----------------|
| rs1042522 | 0        | BstUI  | GTGGAAAGGAAAATTCCAT | GCCAGGCATTGAAGTCTCAT |
| rs1800371 | -76      | MspI   | GACCTGTGGGAAGCGAAAAT | GAGCGCCTCGGCAATTCT |
| rs17878362 | -172     | BstUI  | GTGGAAAGGAAAATTCCAT | GCCAGGCATTGAAGTCTCAT |

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**ARG72PRO POLYMORPHISM BASED ON THE RESULTS PROVIDED BELOW.**

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Although patients were older than controls in our study, the controls were older than the average age of diagnosis and were still free of any signs of POAG. Cases were screened for the presence of the 3 most frequent mutations in MYOC: Gly364Val, Gln368Stop, and Tyr437His and no carriers were detected for these mutations.

We examined genotypic and allelic frequencies for the Arg72Pro SNP (rs1042522) within p53 in our cohort, which were in Hardy-Weinberg equilibrium in both POAG patients and controls. There is a significant difference in genotypic frequencies for rs1042522 between POAG patients and controls ($\chi^2 = 9.56, p=0.008$; Table 3). There were 49% homozygotes for arginine, 43% heterozygotes and 8% homozygotes for proline among controls as compared to 65% homozygotes for arginine, 29% heterozygotes and 6% homozygotes for proline among POAG patients. The allelic frequencies were 85% for arginine and 15% for proline in those with a positive family history compared to 77% for arginine and 23% for proline in those without a family history. Although the frequency of the arginine allele was slightly elevated, it was not significant.

We wondered if the association would be stronger in POAG patients with a positive family history of POAG. The allelic frequencies were 85% for arginine and 15% for proline in those with a positive family history compared to 77% for arginine and 23% for proline in those without a family history. Although the frequency of the arginine allele was slightly elevated, it was not significant.

Next, we examined rs17878362 which is a 16 bp Ins/del polymorphism located in intron 3) did not result in a haplotype that had a stronger effect on POAG risk than the Arg72Pro coding SNP located in exon.

## DISCUSSION

It is well recognized that POAG is a multi-factorial disease and it is expected that multiple genes will contribute to its susceptibility. The goal of this study was to examine the role of the p53 Arg72Pro SNP (rs1042522) in susceptibility to POAG. In our cohort, we have detected evidence for an association between the arginine variant and increased risk of POAG. This association was not affected by differences in gender or age between cases and controls in our sample. The inclusion of rs17878362 (an Ins/del polymorphism located in intron 3) did not result in a haplotype that had a stronger effect on POAG risk than the Arg72Pro coding SNP located in exon.

### Table 2. Subject characteristics.

| Demographic features | Controls | All POAG | POAG-HTG | POAG-NTG |
|----------------------|----------|----------|----------|----------|
| N                    | 167      | 191      | 139      | 52       |
| Females              | 63%      | 48%      | 42%      | 65%      |
| Age at Inclusion     | 60.3±12.0| 67.5±12.5| 66.6±12.6| 69.8±12.0|
| Age at Diagnosis     | 58.8±18.9| 58.1±20.7| 58.1±20.7| 60.9±12.5|
| Family history of POAG | 18%    | 35%      | 36%      | 31%      |

### Table 3. Genotypic and allele frequencies in POAG patients and controls for rs1042522.

| Genotype/Allele | Controls | All POAG | POAG-HTG | POAG-NTG |
|----------------|----------|----------|----------|----------|
| G/G            | 0.49     | 0.65     | 0.64     | 0.69     |
| G/C            | 0.43     | 0.29     | 0.29     | 0.29     |
| C/C            | 0.08     | 0.06     | 0.07     | 0.02     |
| G (Arg)        | 0.71     | 0.80     | 0.78     | 0.84     |
| C (Pro)        | 0.29     | 0.20     | 0.22     | 0.16     |
4. In this study, patients and controls were matched for ethnicity to avoid confounding due to population stratification. All subjects were Caucasian and a majority resided in the same geographical location and represent the Appalachian population. Moreover, the allele frequencies for the Arginine and Proline variants in our controls were similar to the allele frequencies in Caucasian samples from the Hapmap data.

Our results are in contrast to the initial study by Lin et al. [15] where increased frequency of the proline allele (rs1042522) was detected in POAG cases compared to controls. One of the reasons for the discrepancy may be the difference in ethnic populations in each study. According to the Hapmap data, the frequency of the arginine allele (G) and proline allele (C) are 0.51 and 0.49, respectively, in the Chinese population, and 0.77 and 0.23 in the Caucasian population of European origin. The allele frequencies among controls in our cohort are similar to those from the Hapmap data. Although Acharya et al. [16] did not detect a significant difference in an Indian population; they observed a trend for increased frequency of the arginine allele (rs1042522) and the Del allele (rs17878362). Interestingly, in a Caucasian population from England, Ressiniotis et al. [18] observed increased frequency of the arginine allele in POAG cases compared to controls in subjects with the 16 bp insertion allele (rs17878362). However, the finding of the lack of an association between this SNP (rs1042522) and POAG in the sample by Dimasi et
which is contrary to our results can not be easily explained. p53 is one of the key regulators of apoptosis. It can either arrest cell cycle progression in the late G1 phase, thus allowing the DNA to be repaired before replication, or induce apoptosis, leading to cell death. This polymorphism occurs in the proline-rich domain of p53, which is necessary for the protein to fully induce apoptosis. Dumont et al. [19] found that the Arg72 variant had up to 15 fold increased apoptotic ability compared with the Pro72 variant in both inducible cell lines and cells with endogenous p53 homozygous for each variant. They suggested that at least one source of this enhanced apoptotic potential is the greater ability of the Arg72 variant to localize to mitochondria; this localization was accompanied by release of cytochrome c into the cytosol. Another study had noted that Pro72 variant was a stronger inducer of transcription than the Arg72 variant, whereas the Arg72 variant induced apoptosis faster and was a more potent suppressor of transformation than the Pro72 variant [20]. Thus, it is possible that the finding of an association between increased risk of POAG in those homozygous for the Arg72 variant may be due to increased susceptibility of retinal ganglion cells to apoptosis. Interestingly, Dimasi et al. [17] had suggested that a genetic mechanism favoring apoptosis would have a greater role in NTG in which raised IOP is not present. In our cohort, we observed even higher frequencies of the Arginine allele in POAG cases with NTG although this must be interpreted with caution as the number of NTG cases is relatively small.

Further studies consisting of large independent samples with sufficient statistical power for detection of an association and a more detailed analysis of genetic variations within p53 are warranted in order to determine if the association is with Arg72Pro or other alleles in linkage disequilibrium and to determine its role in the pathogenesis of POAG.

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| Genotype/Allele | Controls | All POAG | POAG-HTG | POAG-NTG |
|-----------------|----------|----------|----------|----------|
| Del/Del         | 0.70     | 0.76     | 0.77     | 0.74     |
| Del/Ins         | 0.26     | 0.22     | 0.21     | 0.26     |
| Del/Ins         | 0.04     | 0.02     | 0.02     | 0.00     |
| Del             | 0.83     | 0.87     | 0.87     | 0.87     |
| Ins             | 0.17     | 0.13     | 0.13     | 0.13     |
| Rs17878362/Ins  | 0.04     | 0.03     | 0.03     | 0.03     |
| Rs1042522/Del   | 0.69     | 0.78     | 0.77     | 0.81     |
| Rs1042522/Ins   | 0.14     | 0.10     | 0.11     | 0.07     |
| Rs1042522/Ins   | 0.02     | 0.02     | 0.01     | 0.03     |
| Rs1042522/Ins   | 0.15     | 0.10     | 0.11     | 0.09     |
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