Glaucoma is defined as a group of progressive optic neuropathies characterized by a steady loss of retinal ganglion cells and consequently visual field defects. The number of people with glaucoma is about 60 million worldwide and will exceed 110 million by 2040 [1]. The pathomechanisms that lead to retinal ganglion cell (RGC) death are not fully understood. High intraocular pressure (IOP) is the main risk factor, but in the case of normal tension glaucoma, glaucomatous nerve degeneration occurs although the IOP is within normal limits.

Thus, other factors, such as ocular blood flow [2], inflammation [3], and genetics, must be considered. A positive family history for glaucoma is known to increase individual risk substantially [4]. There is no clear Mendelian inheritance pattern, but some portion of glaucoma is caused by defects in distinct genes, for example, myocilin [5]. The genetics of the larger portion of glaucoma is more complex, and a vast number of gene variants have been implicated [6].

Apoptosis or programmed cell death is a complex process, which ultimately leads to cellular degradation [7]. In glaucoma, several studies emphasized the role of apoptotic factors, such as apoptosis stimulating fragment (FAS) [8], BCL2-associated X protein (BAX) [9], and v-akt murine thymoma viral oncogene homolog 1 (Akt1) [10]. The binding of FAS ligand (FASL) to FAS forms the death-inducing signaling complex, which triggers apoptosis. FAS ligand (FASL) deficiency protects RGCs from cell death [10]. AKT is a serine-threonine protein kinase, which affects the components of the intrinsic cell death machinery [11,12]. The BAX protein is a member of the BCL2 family and has proapoptotic properties by antagonizing BCL2 [13]. Recently, C*HSDGIC*, a novel cyclopeptide from the cyclization of pituitary adenylate cyclase-activating polypeptide, was shown to inhibit apoptosis in retinal ganglion cells by decreasing BAX and increasing BCL2 [14].

For this candidate gene analysis, we selected polymorphisms that had already been demonstrated to alter gene expression or the activity of apoptosis-related genes [15-18]. Identifying the associations of gene polymorphisms with primary open angle glaucoma (POAG) may enhance our understanding of the pathogenesis and may pave the way for future neuroprotective therapies. In addition, a more precise individual risk profile can provide assistance for screening and foreseeing the progression rate of the condition. Therefore, we investigated a hypothesized association between apoptosis-related functional gene polymorphisms and POAG.

**METHODS**

In the present institutional, retrospective case-control study we investigated a total of 668 unrelated Caucasian subjects comprising 334 patients with POAG, and 334 control subjects. In each group 187 females and 147 males were involved. The mean age of patients with POAG was 74.1 ± 8.1 years and 73.2 ± 7.8 years in control subjects, respectively. All participants were of Caucasian origin, living in the same geographical area and were seen at the local Department of Ophthalmology, Medical University of Graz. The study was approved by the Institutional Review Board of the Medical Department of Ophthalmology, Medical University of Graz.
University of Graz and followed the principles of the Declaration of Helsinki. The study adhered to the ARVO statement on human subjects. Prior to enrollment, written informed consent was obtained from all participants.

All patients underwent slit-lamp biomicroscopy, testing for best-corrected visual acuity, Goldmann applanation tonometry, gonioscopy, and standard automated perimetry (Interzeag Octopus 500, program G2) or, in cases of profoundly decreased visual acuity, Goldmann perimetry. In all patients, photographs of the optic discs were taken.

POAG was defined by the IOP before the initiation of a pressure-lowering therapy of at least 21 mmHg, an open anterior chamber angle, optic disc changes characteristic of glaucoma (notching, thinning of the neuroretinal rim, increased cup/disc ratio in relation to the optic disc size), visual field defects characteristic of glaucoma (inferior or superior arcuate scotoma, nasal step, paracentral scotoma), and the absence of conditions that lead to secondary glaucoma. A narrow angle was defined as Schaffer’s grade 2 or less and was excluded with gonioscopy.

The control group consisted of 334 unrelated patients with no morphological or functional damage indicative of primary or secondary open angle or angle closure glaucoma. The control subjects had been admitted to the department for cataract surgery. All participants were Caucasians from the same geographic area (southern Austria).

**Genotype determination:** Venous blood was collected in 5 ml EDTA tubes. DNA was isolated from peripheral lymphocytes using a QIAamp DNA blood mini-kit (Qiagen, Venlo, Netherlands) following the manufacturer’s protocol and stored at −20 °C. Genotype determination was performed using high-resolution melting curve analysis on the LightCycler® 480 PCR system (Hoffmann-La Roche, Basel, Switzerland). The samples were amplified in duplicate 20 µl reactions using the Light Cycler 480 High Resolution Melting Master kit (Roche Diagnostics, Wien, Austria) and analyzed on an LC480 instrument I (Roche Diagnostics GmbH, Mannheim, Germany). The final reaction mix contained 1X Master Mix, 3 mM MgCl₂, 4 µM forward and reverse primer, and 50 ng of genomic DNA. Primer sequences are shown in Table 1.

| Gene | SNP   | Primer (5’-3’)                  |
|------|-------|---------------------------------|
| AKT1 | rs1130233 | F: AGCTGTCTTCCACCTGTMML       |
|      |       | R: TCTCGAGTGCGAGTGTGML         |
| BAX  | rs4645878 | F: ACCCTGCCGAAAACCTAATT   |
|      |       | R: GAGCATCTCCCCGATAAGTC        |
| FAS  | rs2234767 | F: CTTATACCCTCCTTTTTGTGTC     |
|      |       | R: GGCTTGTCCTGTCCACCT         |
| FASL | rs763110 | F: CTTGGCAAAACATGAAATG       |
|      |       | R: ACCCACTTTAGAAAATTAGATCA     |

Statistical analysis: Descriptive statistics were used to calculate the frequencies and percentages of discrete variables. Continuous data are given as mean ± standard deviation (SD). Means were compared using the Mann–Whitney test. Proportions of groups were compared with a chi-square test. The criterion for statistical significance was p value less than 0.05. The Bonferroni correction was used to adjust for multiple testing. Hardy–Weinberg equilibrium was calculated using HW Diagnostics-Version 1.beta (Fox Chase Cancer Center). Statistical analysis was performed using the SPSS statistical package (SPSS, version 17.0, Chicago, IL). Power calculation was done using PS Power and Sample Size Calculation software version 2.1.30.

**RESULTS**

Our study included 334 patients with POAG and an equal number of control subjects. In each group, 187 women and 147 men were included. The mean age of the patients with POAG and the control subjects was 74.1±8.1 years and 73.2±7.8 years, respectively.

The observed genotype distributions did not deviate from those predicted by Hardy–Weinberg equilibrium. Table 2 shows the genotype and minor allele frequencies of the
investigated polymorphisms in the patients with POAG and
the control subjects. Genotype AA of AKT1 rs1130233 was
significantly more common in POAG, but this significance
did not remain after the Bonferroni adjustment. Therefore,
no significant difference in either the genotype or allelic
frequencies of any investigated gene variant was found.
Quantitative trait analysis with age at presentation, mean
defect in visual fields, vertical cup/disc ratio, or intraocular
pressure did not reveal any association (p>0.05).

The present study had a statistical power of 0.80 to detect
an odds ratio of 1.86 at a significance level of 0.05.

DISCUSSION

To the best of our knowledge, the present study is the first to
investigate a hypothesized association between POAG and
functional apoptosis-related gene polymorphisms. Geno-
types of four functional apoptosis-related polymorphisms
were analyzed in 334 patients with POAG and 334 controls
matched for age and sex. After correction for multiple testing,
the allelic frequencies and genotype distribution did not differ
significantly between the groups.

AKT inhibits apoptosis by phosphorylation and, thus,
inhibition of transcription factors of the forkhead family,
which trigger programmed cell death by inducing FASL
[19]. Furthermore, AKT1 influences nerve cell plasticity and
development [20]. The influence of rs1130233 on the expres-
sion of AKT1 has been demonstrated in lymphoblasts [21].
Although gene variants of AKT1 have been shown to affect
apoptosis in a cell model [18], we could not find an associa-
tion with POAG.

Gene polymorphisms have been shown to affect nuclear
protein binding affinity and thus the transcription of BAX
[22]. Mice deficient in BAX were protected from retinal
ganglion cell death suggesting a major role of BAX in glau-
coma development [23]. However, we did not find an associa-
tion of the BAX gene polymorphism rs4645878 with POAG,
which is in line with the findings of a recent meta-analysis in
chronic lymphatic leukemia [24]. Despite the functionality
of this polymorphism, it was not shown to alter the predisposi-
tion of POAG or chronic lymphatic leukemia, two diseases in
which apoptosis plays a pivotal role.

Binding of FAS to FASL leads to caspase 8 activation
and is a key mechanism of normal tissue homeostasis [25].
Polymorphisms in FAS and FASL have been found to be
associated with various inflammatory diseases [26-28] and
cancer [29-31]. Moreover, the expression of FAS and FASL
is altered in neurodegenerative disorders. The FAS/FASL
pathway has already been demonstrated to play a critical
role in mediating beta-amyloid-induced death of neurons in

| Gene | Mutation | POAG | Controls | P-value | Odds ratio                  |
|------|----------|------|----------|---------|-----------------------------|
| Akt1 | G/G      | 216  | 206      | 0.422   |                             |
|      | rs1130233| A/G  | 99       | 116     | 0.159                       |
|      | MAF      | A/A  | 19       | 8       | 0.031 0.80 (0.62–1.05)       |
|      |          |      | 0.21     | 0.2     | 0.81                          |
| Bax  | G/G      | 259  | 254      | 0.647   |                             |
|      | rs4645878| A/G  | 66       | 75      | 0.393                       |
|      | MAF      | A/A  | 9        | 5       | 0.28 1.01 (0.73–1.40)        |
|      |          |      | 0.13     | 0.13    | 0.87                          |
| Fas  | G/G      | 275  | 265      | 0.326   |                             |
|      | rs2234767| A/G  | 54       | 65      | 0.266                       |
|      | MAF      | A/A  | 5        | 4       | 0.737 1.16 (0.81–1.65)       |
|      |          |      | 0.1      | 0.11    | 0.36                          |
| FasL | G/G      | 132  | 150      | 0.159   |                             |
|      | rs763110 | A/G  | 159      | 145     | 0.277                       |
|      | MAF      | A/A  | 43       | 39      | 0.637 0.87 (0.70–1.08)       |
|      |          |      | 0.37     | 0.33    | 0.27                          |

Absolute numbers of patients and controls are shown. Rs-numbers of the investigated polymorphism are depicted below the gene name. Minor allele frequencies (MAF) are indicated in the bottom line. Odds ratios are given with 95% confidence interval in parentheses.
Alzheimer’s disease [32,33]. Interestingly, in a study that used enzyme-linked immunosorbent assay (ELISA), FASL levels were not significantly altered in patients with POAG [34], and with multiplex beads analysis, the FASL levels were below the limits of detection in aqueous humor [35]. However, other studies identified the upregulation of FAS/FASL in glaucoma [10,36,37]. Due to these conflicting results, the role of FAS/ FASL in glaucoma remains elusive. Still, our findings do not support the pivotal influence of FAS/FASL on glaucoma pathogenesis.

The following potential limitations should be kept in mind when interpreting our results. First, only a small number of single nucleotide polymorphisms (SNPs) were investigated in the present study. We cannot rule out associations with other polymorphisms of the investigated genes. A tagged SNP approach would be a possible strategy here, but it was beyond the scope of this survey. All investigated SNPs had already been demonstrated to be functional. Second, as genetic polymorphisms have been shown to vary between populations, our findings do not necessarily apply to populations other than Caucasian. In conclusion, our findings suggest that the investigated gene polymorphisms are unlikely to be major risk factors for POAG in Caucasian patients.

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