Methylenetetrahydrofolatereductase (MTHFR) 677C>T polymorphism and open angle glaucoma

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Purpose: Increased plasma homocysteine levels have been found in patients with primary open angle glaucoma (POAG) and glaucoma secondary to pseudoexfoliation syndrome (PEX). A common polymorphism of the methylenetetrahydrofolatereductase (MTHFR 677C>T) leads to moderately elevated plasma homocysteine levels particularly under conditions of impaired folate status. It was the aim of this study to investigate a hypothesized association between this polymorphism and the presence of either POAG or PEX.

Methods: The present retrospective case-control study included a total of 553 participants comprising 204 patients with POAG, 138 patients with PEXG, and 211 control subjects. Genotyping for the MTHFR 677C>T polymorphism was performed by polymerase chain reaction (PCR).

Results: No significant difference in the genotype distribution of the MTHFR 677C>T polymorphism was found between control subjects and patients with POAG or PEXG. The prevalence of the MTHFR 677TT genotype was 6.9% in patients with POAG, 11.6% in patients with PEXG, and 9.5% in control subjects.

Conclusions: The present data suggest that the MTHFR 677C>T polymorphism itself is not a major genetic risk factor for POAG and PEXG in a central European population.

Primary open angle glaucoma (POAG; OMIM 137760) is a major cause of blindness throughout the world [1,2]. It is defined as a slowly progressive multifactorial optic neuropathy with characteristic loss of retinal ganglion cells and atrophy of the optic nerve leading to visual field defects. Elevated intraocular pressure is the most relevant risk factor, but vascular, immunological, or neurotoxic factors have also been implicated in the pathophysiology of POAG [3]. Pseudoexfoliation syndrome (PEX; OMIM 177650) is characterized by the accumulation of fibrillar material in ocular and extraocular tissues. This leads to elevated intraocular pressure and consequent glaucomatous damage of the optic nerve (PEXG) in approximately 50 percent of patients [4]. PEXG is the most common identifiable cause of secondary open angle glaucoma. Despite great efforts, pathogenesis of both POAG and PEXG remains elusive and various contributing factors have been suggested. In recent years the role of homocysteine has been investigated in context with POAG and PEXG. Homocysteine, a sulfur-containing amino acid synthesized during the metabolism of methionine, is a well-known independent risk factor for vascular disease [5]. In neurodegenerative diseases, the role of increased plasma homocysteine levels has been controversial [6,7]. Homocysteine induces apoptotic cell death of retinal ganglion cells [8] and also exerts gliotoxic effects, thus representing a hypothetical risk factor for POAG [9]. Furthermore, homocysteine causes dysregulation of matrix metalloproteinases and their inhibitors [10], which has been implicated in the pathogenesis of PEXG [11]. This has led to the assumption that homocysteine may also participate in the pathogenesis of PEXG. Bleich and coworkers reported an association between elevated plasma homocysteine levels and POAG and PEXG [12]. However, subsequent studies investigating the role of plasma homocysteine levels in POAG were unable to confirm this finding [13,14], whereas studies investigating plasma homocysteine levels in patients with PEX revealed controversial results [14-18]. Plasma homocysteine concentrations are influenced by both environmental and genetic factors, such as the methylenetetrahydrofolatereductase (MTHFR; OMIM 607093) 677C>T polymorphism, the most common genetic determinant of elevated levels of plasma homocysteine [19].

MTHFR catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which is required for remethylation of homocysteine to methionine. In 1991, Kang and coworkers discovered a thermolabile variant of MTHFR with 50% residual activity, characterized by a C>T substitution at nucleotide 677 (MTHFR 677C>T) [20,21]. Under conditions of impaired folate status, the MTHFR 677TT genotype leads to moderately elevated plasma homocysteine levels [22].

Junemann and coworkers found an increased frequency of the 677C>T polymorphism of MTHFR in patients with POAG, but not in patients with PEXG [23]. Only recently
Turaci and coworkers reported no significant difference of the 677C>T polymorphism of the MTHFR between PEX patients and controls [18]. The number of patients enrolled in these studies, however, was rather small.

The present study was designed to investigate the role of the MTHFR 677C>T polymorphism in a larger cohort of patients with POAG and PEXG.

**METHODS**

In our case-control study, we investigated a total of 553 Caucasian subjects comprising 204 patients with POAG, 138 patients with PEXG, and 211 control subjects. All participants were seen at the Department of Ophthalmology, Medical University of Graz, and they gave written informed consent prior to enrollment. The study was conducted in accordance with the standards of the local Ethics Committee and the National Gene Technology Act.

All patients underwent slit lamp biomicroscopy, testing for best corrected visual acuity, Goldmann applanation tonometry, gonioscopy, and standard automated perimetry (Interzeag Octopus 101, programme G2) or, in cases of profoundly decreased visual acuity, Goldmann perimetry. Photographs were taken of every patient’s optic discs.

POAG was defined by the following criteria: an intraocular pressure before initiation of a pressure-lowering therapy of at least 21 mm Hg, an open anterior chamber angle, optic disc changes characteristic for glaucoma (notching, thinning of the neuroretinal rim, increased cup/disc ratio in relation to the optic disc size), visual field defects characteristic for glaucoma (inferior or superior arcuate scotoma, nasal step, paracentral scotoma), and absence of conditions leading to secondary glaucoma. A diagnosis of PEXG was made if the patient met the following criteria: intraocular pressure before initiation of a pressure-lowering therapy of at least 21 mm Hg, an open anterior chamber angle, optic disc changes characteristic for glaucoma, and the presence of typical pseudoxfolliation material on the anterior lens capsule. The control group consisted of 211 unrelated patients with no morphological or functional damage indicative for primary or secondary open angle or angle closure glaucoma. Control subjects were admitted to our department for cataract surgery. Medical history concerning arterial hypertension, diabetes mellitus, cardiovascular events, and recent medication was obtained from all participants. All participants were Caucasians from the same geographic area (southern Austria).

Genomic DNA was extracted from peripheral blood lymphocytes by standard techniques. MTHFR mutation analysis was performed by polymerase chain reaction-restriction fragment length polymorphism (RFLP) according to the technique of Froos et al. [21], by a technician unaware of the status of the DNA sample.

**Statistical analysis:** Descriptive statistics were used to calculate frequencies and percentage of discrete variables. Proportions of groups were compared by the $\chi^2$ test. Odds ratio (OR) and 95% confidence interval (95% CI) were calculated by logistic regression. The criterion for statistical significance was $p<0.05$. Statistical analysis was done using SPSS 10.0 for windows (SPSS, version 10.0, Chicago, IL). Power analysis was done using PS Power and Sample Size Calculation software version 2.1.30.

**RESULTS**

Our study included 204 patients with POAG (129 females, 75 males), 138 patients with PEXG (84 females, 54 males), and 211 control subjects (131 females, 80 males). The mean age of patients with POAG was 73.1 years, 75.7 years in patients with PEXG, and 73.1 years in control subjects, respectively. Demographic data are shown in Table 1.

No significant differences in either genotype distribution or allele frequencies of the MTHFR 677C>T polymorphism were found between patients with POAG or PEXG and control subjects (Table 2). Presence of the MTHFR 677T-allele was associated with an odds ratio of 0.71 (95% CI 0.48-1.04) for POAG, whereas an odds ratio of 0.91 (95% CI 0.59-1.39) was calculated for patients with PEXG. The observed genotype distributions did not deviate from those predicted by the Hardy-Weinberg equilibrium, and for control subjects were in Hardy-Weinberg equilibrium, and for control subjects were in Hardy-Weinberg equilibrium.

**Table 1.** Demographic data of patients and controls. Descriptive statistics of POAG patients, PEXG patients, and control subjects including prevalence of arterial hypertension, diabetes mellitus, history of myocardial infarction, and history of stroke are presented. The gender and history data are presented as counts with percentages in parentheses. The cases were compared to the controls using the $\chi^2$ test. The asterisk indicates a $p<0.05$ for the comparison to controls.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patients with POAG</th>
<th>Patients with PEXG</th>
<th>Control subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients (patients)</td>
<td>204</td>
<td>138</td>
<td>211</td>
</tr>
<tr>
<td>Age (years)</td>
<td>Range</td>
<td>Range</td>
<td>Range</td>
</tr>
<tr>
<td></td>
<td>73.1±10.3</td>
<td>75.7±7.3</td>
<td>73.1±9.7</td>
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<td></td>
<td>37.1-90.3</td>
<td>50.3-91.6</td>
<td>37.4-90.3</td>
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<tr>
<td>Gender (Female)</td>
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<td>84 (60.9)</td>
<td>131 (62.1)</td>
</tr>
<tr>
<td>Arterial hypertension</td>
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<td>80 (60.0)</td>
<td>110 (52.1)</td>
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<tr>
<td>Diabetes mellitus</td>
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<td>18 (13.0)*</td>
<td>46 (21.8)</td>
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<tr>
<td>History of myocardial</td>
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<td>7 (5.1)</td>
<td>12 (5.7)</td>
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<tr>
<td>infarction</td>
<td>20 (9.8)</td>
<td>14 (10.1)</td>
<td>12 (5.7)</td>
</tr>
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</table>

**Table 2.** Distribution of the MTHFR 677C>T genotype. The table presents genotype distribution and frequency of the MTHFR 677T allele in patients with POAG, patients with PEXG, and control subjects. Using the $\chi^2$ test, no statistically significant differences were found. The 677T-allele frequencies were 0.24, 0.30, and 0.30 for POAG patients, PEXG patients, and controls subjects, respectively.
the range of those reported for Caucasian populations in central Europe [24-28].

The present study had a statistical power of 0.80 to detect an odds ratio of 2.25 for the MTHFR 677TT genotype in patients with POAG and an odds ratio of 2.42 for the MTHFR 677TT genotype in patients with PEXG.

**DISCUSSION**

Elevated levels of plasma homocysteine have been reported inconsistently in patients with PEXG and POAG. Under conditions of impaired folate status homozygosity for the MTHFR 677C>T polymorphism leads to moderately elevated homocysteine levels. Therefore, this polymorphism itself is debated as a potential genetic risk factor for POAG and PEXG. Indeed, Junemann and coworkers reported a positive association between POAG and the MTHFR 677C>T polymorphism, but not for PEXG. Their study included 76 patients with POAG, 71 patients with PEXG, and 71 control subjects. Genotype distribution of homozygosity (TT) and heterozygosity (CT) was 9% and 49% in patients with POAG and 9% and 41% in patients with PEXG, whereas the control group revealed a distribution of 3% and 34%. The authors thus suggested that the MTHFR 677C>T polymorphism may play a role in the pathogenesis of POAG [23]. Turací et al. [18] found no significant difference in the 677C>T polymorphism of MTHFR between patients with PEX and control subjects. In their study, genotype distribution of homozygosity (TT) and heterozygosity (CT) was 7.9% and 40.8% in patients with PEX (60 patients with PEXG and 16 patients with PEX), whereas the control group (34 subjects) revealed a distribution of 11.8% and 35.3%. In European populations, the prevalence of homozygosity for the MTHFR 677T-allele has been reported to be between 4.0 and 26.4% with increasing prevalence in southern Europe [24]. The MTHFR 677C>T polymorphism has been recently investigated in a cohort of patients with cataract. The genotype distribution of the MTHFR 677C>T polymorphism was found in similar frequency among patients with cataract and control subjects [29].

To date, our study is the largest to investigate the role of the MTHFR polymorphism in POAG and PEXG. Our research found that the prevalence of the MTHFR 677C>T polymorphism did not differ significantly between both study groups and control subjects. We therefore suggest that the MTHFR 677C>T polymorphism itself is not a major genetic risk factor for POAG and PEXG in central Europe.

Plasma levels of homocysteine are influenced by various other factors. Deficiencies of folate, vitamin B12, and B6 account for the majority of cases of hyperhomocysteinemia. In addition, lifestyle factors (e.g., smoking and chronic alcohol intake) and numerous drugs, such as fribates, carbamazepine, phenytoin, antifolates (methotrexate and trimethoprim), and postmenopausal hormone replacement therapy [30,31] as well as polymorphisms of the cystathionine β-synthase and cystathionine β-lyase [32] are known to increase plasma homocysteine levels. Thus, our results do not argue against a potential role of elevated plasma levels of homocysteine in the pathogenesis of POAG and PEXG. In conclusion, no statistically significant difference in the genotype distribution of the MTHFR 677C>T polymorphism was found between patients with POAG or PEXG and control subjects, strongly suggesting that this polymorphism itself is not a major risk factor for the investigated diseases.

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**REFERENCES**