

Short communication

**Only neutral polymorphisms found
in the *TIGR*/myocilin gene of 45 Polish patients
with primary open-angle glaucoma**

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Abstract. The aim of the study was to identify mutations of the *TIGR* gene in Polish patients with primary open-angle glaucoma (POAG) and to define possible genotype-phenotype correlations. The study included 45 patients with a verified diagnosis of POAG. The PCR amplification of all three exons of the *TIGR* gene and screening for the sequence changes by CSGE analysis was done for every patient. The probes with identified heteroduplexes were sequenced. Altogether 315 PCR products were obtained. The CSGE analysis detected 60 possible changes of the sequence in 28 patients. 34 heteroduplexes were chosen for sequencing, including 29 unique changes and 5 changes representative of identical heteroduplexes. Direct sequencing enabled detection of only four different changes in the *TIGR* gene sequence. Three of them: 5'UTR -83G→A (in 14 patients), +227 exon 1 G→A, Arg76Lys (in 14 patients) and +311 exon 3 T→C, Tyr347Tyr (in 4 patients) have already been described in the literature as neutral polymorphisms of the gene. Only one change in the promoter, 5'UTR -126T→C (in 2 patients), has not been described in the literature to date. However, this change does not alter directly the sequence of amino acids in myocilin, so it is difficult to conclude on its pathogenetic role. Thus our study showed only neutral polymorphisms of the *TIGR* gene. This suggests that the patients probably have mutations in other genes, so other loci that predispose to POAG must be analyzed.

Key words: mutations, polymorphisms, primary open-angle glaucoma, *TIGR*/myocilin gene.

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Primary open angle glaucoma (POAG) is the main cause of irreversible blindness in developed countries. Therefore there are many attempts to allow the earliest possible identification of patients at risk of glaucoma development, because it can offer a chance of early prophylaxis.

Genetic linkage analyses have shown that there are six different loci linked to predisposition to POAG (OMIM). In one of them, *GLC1A* on chromosome 1q21-q31, STONE et al. (1997) identified in 1997 the *TIGR* gene that codes for the myocilin protein and proved that its mutations are responsible for development of POAG (OMIM 601652). This gave rise to numerous studies looking for *TIGR*/myocilin gene mutations that predispose to POAG (ALWARD et al. 1998, FAUCHER et al. 2002, FINGERT et al. 1999, MANSERGH et al. 1998).

The aim of this study was to identify mutations of the *TIGR*/myocilin gene in Polish patients with POAG and to define possible genotype-phenotype correlations.

We studied 45 unrelated patients (25 males and 20 females) who presented to the Glaucoma Clinic, Department of Ophthalmology, University of Medical Sciences, Poznań. In this group of patients there were 21 sporadic cases and 24 cases with affected I^o and II^o relatives. In every patient the diagnosis of POAG was proved by a thorough ophthalmologic examination. The inclusion criteria were: (1) initial intraocular pressure higher than 21 mmHg in repeated measurements by a Goldmann aplanation tonometer; (2) Shaffer grade 3 or 4 during gonioscopic examination of iridocorneal angle; (3) progressive glaucomatous visual field changes in repeated examination done by Humphrey static perimeter, and (4) glaucomatous changes of the optic nerve head in stereoscopic indirect ophthalmoscopy. This was one of the biggest groups of families in Europe analyzed towards the *TIGR*/myocilin gene mutations (BREZIN et al. 1998, VAZQUEZ et al. 2000).

Participating patients gave their informed consent to be included into the study. DNA of patients was isolated from peripheral blood lymphocytes. For every patient, the PCR amplification of all three exons of the *TIGR*/myocilin gene was done. Primers and conditions used for PCR amplification are shown in Table 1.

The screening of the PCR products was done using conformation-sensitive gel electrophoresis (CSGE) analysis. The probes with identified heteroduplexes were sequenced with an automatic DNA sequencer. For every patient, PCR was done seven times, to amplify the coding regions of the *TIGR* gene, promoter sequence, and flanking sequences of introns as well. Altogether 315 PCR products were obtained. The CSGE analysis allowed to detect: 32 heteroduplexes in exon 1, 8 heteroduplexes in exon 2, and 20 heteroduplexes in exon 3. Altogether 60 possible changes of the sequence were identified in 28 patients. 34 heteroduplexes were chosen for sequencing, including 29 unique changes and 5 changes representative of numerous identical heteroduplexes in different patients.

Table 1. Primers and conditions used for PCR amplification

Exon	Primer	Primer's sequence 5'→3'	Size of PCR product	Hybridization temperature [°C]
1	Ex1P1	AATCTTgCTggCagCgTg	389 bp	60
1	Ex1P2	AgCTggATTCATTgggAC	389 bp	60
1	Ex1P3	TgCAATgAgCTTCTTCTg	421 bp	58
1	Ex1P4	TCCAACCTCTCTgCTTTggg	421 bp	58
1	Ex1P5	CagTCATCCATAAATTAC	485 bp	56
1	Ex1P6	ATATCACCTgCTgAACTC	485 bp	56
2	Ex2P7	CATAgTCAATCCTTgggC	288 bp	54
2	Ex2P8	GggAACAgAgAgAgAgAg	288 bp	54
3	Ex3P9	ggATTAAgTgTggTgCTgCg	454 bp	62
3	Ex3P10	AATACgggAACTgTCggTgg	454 bp	62
3	Ex3P11	AgAAggAAATCCCTggAg	420 bp	60
3	Ex3P12	CATAAgTgACCATgTTCAAg	420 bp	60
3	Ex3P13	ATTgACTACAACCCCTG	450 bp	59
3	Ex3P14	GCTTgTggTAACCATgTAAC	450 bp	59

Direct sequencing allowed us to detect only four different changes in the *TIGR* gene sequence. Three of them: 5'UTR -83G→A (in 14 patients), +227 exon 1 G→A, Arg76Lys (in 14 patient) and +311 exon 3 T→C, Tyr347Tyr (in 4 patients) have already been described in the literature as neutral polymorphisms of the *TIGR* gene (FAUCHER et al 1999, FINGERT 1999).

Only one change in the promoter, 5'UTR -126T→C (in 2 patients), has not described been in the literature to date. However, because this change does not alter directly the sequence of amino acids in myocilin, it is very difficult to conclude on its pathogenetic role. There are, however, some papers (COLOMB et al. 2001) showing an important role of *TIGR* gene promoter polymorphisms. Anyway, our study on patients with POAG showed mainly known neutral polymorphisms of the *TIGR*/myocilin gene. The role of the change in promoter sequence that has not been described yet, will be analyzed by checking its prevalence in a bigger control group of healthy persons.

We suppose that the lack of *TIGR*/myocilin mutations in our study may be caused by the relatively small number of patients, although several other studies made on a comparable or bigger number of patients showed that the prevalence of *TIGR*/myocilin mutations is 4-8% in sporadic cases (ALWARD et al 1998, FAUCHER et al. 2002, FINGERT et al. 1999, VAZQUEZ et al. 2000, YOON et al. 1999) and even 22% in familial cases of POAG (FAUCHER et al. 2002).

Exclusion of *TIGR*/myocilin gene mutations in the group of Polish patients with POAG means that they probably harbor mutations in other genes. This paves the way to studies of other loci that predispose to POAG, such as optineurin (*GLC1E*) (REZAIIE et al. 2002) or the *CYP11B1* gene (VINCENT et al. 2002).

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