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Frequency analysis of single-nucleotide polymorphisms 1151 A>G and 1021T/C of MYOC gene with development of primary open-angle glaucoma in Uzbek population

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Abstract: Glaucoma, one of the leading causes of irreversible blindness worldwide, is characterized by the progressive loss of retinal ganglion cells and their axons. Primary open-angle glaucoma (POAG) is the most common form. The peculiarity of POAG flow is practically asymptomatic onset of the disease; new diagnostic methods, including molecular-genetic methods of examination, are of great importance. Mendeleev's genetic approaches allowed to identify 15 genes and 31 locuses that cause glaucoma development. Myocilin is the first gene to be identified. The conducted researches have shown that heterozygous genotypes by the studied polymorphisms of MYOC gene are not reliably met in the sample of patients of Uzbek population with POAG in comparison with control.

Keywords: Primary open-angle glaucoma, intraocular pressure, MYOC gene polymorphism.

Introduction:

Glaucoma, one of the leading causes of irreversible blindness worldwide, is characterized by the progressive loss of retinal ganglion cells and their axons. Primary open-angle glaucoma (POAG), as the most common form,[1] is a multifactorial disease, belongs to the group of pathologies with hereditary predisposition, to the etiology of which, along with environmental factors, genetic components make a significant contribution [2.] According to different authors, the share of genetically determined cases is from 21 to 50% [3,4,5].

Mendeleev's genetic approaches made it possible to identify 15 genes and 31 locuses that cause glaucoma development.

Myocilin is the first identified gene to mutation in both juvenile and primary open-angle glaucoma. The myocylin gene located on the long shoulder of the 1st chromosome (locus 1q24.3-q25.2) was first described by E.M. Stone in 1997. More than 70 mutations have now been identified in the myocylin gene (MYOC/TIGR/GLC1A). Its expression was detected in the trabecular network of the anterior chamber angle, ciliary body, sclera, chorioidea, cornea, iris, retina, helmet channel, and postlaminar axons of the optic nerve. The protein product of the gene is secretory protein myocylin. The greatest amount of protein is found in intercellular matter among collagen fibers and in cells of the trabecular zone, sclera and cornea. The mutant protein becomes insoluble and accumulates inside the cells of the trabecular network, causing their dystrophic changes and subsequent death by apoptosis. The consequence of these processes is increased resistance to the outflow of intraocular fluid and increased intraocular pressure (IOP). Since myocylin is also expressed in ganglion cells of the retina, astrocytes, mutations in the gene can directly lead to their apoptosis without increasing the intraocular pressure, and as a result, cause a decrease in the mechanical stability of the lattice plate. The most studied variations of myocilin gene are Q368X, Gln368Stop, the most frequently encountered P370L, 3Pro370Leu[14].

The aim of this study was to analyze the frequency of possible association of single-nucleotide polymorphisms 1151 A>G and 1021T/C of MYOC gene with the development of primary open angle glaucoma in Uzbek population.

Research material and methods:

The study included 118 patients (118 eyes) with POAG at the age of 40 to 83 years (the average age of the patients was 57±2.92 years) who were examined and treated in the RSSPMCEM. Among them, 49 were women and 69 were men. The distribution of patients by stages was as follows: with a developed stage of POAG there were 48 eyes,

with a far-reaching stage - 62 eyes, terminal stage was detected on 8 eyes. All the patients underwent computer perimetry in the threshold mode on the perimeter of PTS 1000 Optopol ("Optopol Technology Spolka Akcyjna", Poland), visometry on the phosphorator Topcon CV-5000PRO ("Topcon Corporation", Japan), tonometry for Maklakov, investigation of critical frequency of flicker fusion on the device "Sveto - Test" of "Okulus" company, front-back eye size (VuMAX ("Sonomed Escalon" USA), biomicroscopy on slot lamp HS-7000 ("Huvitz" South Korea), ophthalmoscopy with fundus-lens.

In all patients with POAG and in healthy unrelated donors who made up the control group, PCR genotyping of 1151 A>G and 1021T/C myocylin gene polymorphism was performed in the laboratory of molecular genetics of the Research Institute of Hematology and Blood Transfusion. The blood was taken on an empty stomach from the ulnar vein of the examined patients under sterile conditions. DNA extraction from peripheral blood was performed using a standard set of Ribo-sorb (AmpliSens®, Russia). PCR genotyping of polymorphism 1151 A>G and 1021T/C of myocylin gene was performed using a thermocycler "Applied Biosystems" 2720 (USA), using a test set of "Litech" LLC (Moscow) according to the manufacturers' instructions.

The application package "OpenEpi 2009, Version 2.3" was used as a statistical calculation tool.

Results: As a result of the examination it was revealed that visual acuity in the group of patients with developed stage of POAG ranged from 0.1 to 1.0 (average index - 0.4±0.1), intraocular pressure on Maklakov was within 16.0 - 37.0 mmHg. (mean value - 23±4.7 mmHg), the critical frequency of flicker fusion was 23-39 hertz (mean value - 32±8.9 hertz), the total field of vision was from 90 to 440 degrees (mean value -

263±1.2 hertz), the anterior posterior eye size ranged from 24.49 to 26.06 mm (mean value - 23±2.7 mm) (Table 1). Patients of this group had the following operations:

- Laser iridotomy -2

- non-penetrating deep sclerectomy - 5

- sinusotrabeculectomy-8

- sinusotrabeculectomy with posterior sclera-12 trepania

- deep sinusotrabeculectomy with posterior sclera-20 trepanation

- phacoemulsification with intraocular lens 1 implantation

In the group of patients with far-away stage POAG visual acuity ranged from 0.01 to 0.9 (mean value - 0.2±0.8), intraocular pressure on Maklakov - 14.0-38.0 mmHg. (mean value 24±6.7 mmHg), critical frequency of flicker fusions from 10 to 39 hertz (mean value 28±4.1 hertz), the total field of vision was from 40 to 345 (mean value 210±3.2), anterior to posterior eye size ranged from 21.63 to 29.42 mm (mean value 23±5.3 mm). The following types of operations were performed on this group of patients:

- Laser iridotomy -2

- impermeable deep sclerectomy - 1

- sinusotrabeculectomy with posterior sclera-19 trepanation

- deep sinusotrabeculectomy with posterior trepania sclera-39

- phacoemulsification with intraocular lens 1 implantation

Patients with terminal stage POAG had visual acuity within 0.01-pr. In lucie certa (0.001) intraocular pressure was 19.0 - 25.0 mmHg. (mean value 26±0.5 mmHg), critical frequency of flicker fusions from 8 to 19 Hz (mean value 17±8.7 Hz), total field

of vision was from 15 to 40 degrees (mean value 41±2.5), anterior posterior eye size ranged from 22.25 to 26.89 mm (mean value 24±0.4 mm). The following types of operations were performed on this group of patients:

- sinusotrabeculectomy from posterior trepania sclera-3

- deep sinusotrabeculectomy with posterior sclera-4 trepanation

- deep sinusotrabeculumectomy with posterior sclera trepanation with xenocollagen drainage implantation -1

Table 1

Anatomically-functional indices of the visual organ of patients with POAG (M+m)

Indicators	Developed st. POAG.	A faraway st. POAG.	Terminal st. POAG
Acute vision with maximum correction	0,4±0,1	0.2±0,8 (mean value)	0,001 (mean value)
Intraocular pressure	23±4,7 mmHg (mean value)	24±6,7 mmHg (mean value)	26±5 mmHg (mean value)
Critical Flicker Fusion Frequency	32±8,9 hz (mean value)	28±4,1 hz (mean value)	17±8,7 hz (mean value)
Total field of vision	263±1,2 degrees	210±3,2 degrees (mean value)	41±2,5 degrees (mean value)

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Fore-anterior size of the eye	23±2,7мм (mean value)	23±5,3мм (mean value)	24±0,4мм (mean value)

The MYOC gene 1151A>G and 1021T /C polymorphisms were detected by PCR followed by real-time analysis of DNA and PCR restriction fragment length polymorphisms. After amplification, restriction was performed. For 1151 A>G and 1021T/C MYOC gene polymorphisms no reliable gender differences in allele and genotype frequencies were found, samples of men and women were combined as a common group.

According to the results, the observed genotype frequency distributions in the control group correspond to the theoretically expected distributions, according to the Hardy-Weinberg Distribution Law (HWD). At the same time, in the group of patients with POAG a deviation of the empirical distribution of genotype frequencies from PCB was noted due to a decrease in the observed A/G heterozygosity and homozygosity level of polymorphism 1151 A>G ($p<0.05$). At the same time, the lack of heterozygotes was at D=+0.15 and D=+0.04.

The frequency of adverse alleles G and C of 1151 A>G and 1021T/C polymorphisms in the patient groups was 2.0% and 2.9%, respectively. In the patient group the frequency of these alleles was also low and made 0.0% and 1.0%, respectively.

The frequency of heterozygous A>G and T/C genotypes by the polymorphisms 1151 A>G and 1021T/C in the patients with POAG was 3.9% (2/51) and 5.9% (3/51), respectively. In the control group the frequency of these genotypes was 0.0 and 2.0%, respectively. The frequency of homozygous genotypes A/A and C/C in the studied group of patients was 96.1% and 94.1%, respectively.

Despite this, the frequency of detection of heterozygous polymorphism genotype 1151 A>G among patients was 4 times higher in comparison with conditionally healthy persons. Despite high RR and OR scores, the revealed differences were not statistically significant ($\chi 2<3.8$; $p>0.05$). Differences in the frequency values of heterozygous polymorphism genotype 1021T/C in the studied patient groups and controls were also not statistically significant, with a prevalence among patients ($\chi 2 <3.8$; $p>0.05$; RR=2.9; OR=3.1; 95% CI: 0.3077-30.48).

Conclusion

Since the POAG flow is characterized by an almost asymptomatic onset of the disease, new diagnostic methods, including molecular genetic examination, are important. The conducted researches have shown that heterozygous genotypes by the studied polymorphisms of MYOC gene are not reliably met in the sample of patients of the Uzbek population with POAG in comparison with control.

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