ANALYSIS OF MYOCILIN (MYOC) AND NEUROTROPHIN 4 (NTF4) GENES IN PATIENTS WITH GLAUCOMA IN KAZAKHSTAN

Abstract. Primary Open-Angle Glaucoma being the most common type of glaucoma has a great socio-medical significance and it is a major focus for scientific researching in ophthalmology. In 21-50% of cases the disease is the result of genetic causes and for descendants of people with glaucoma, the risk of this pathology is 10 times higher. Mutations causing Primary Open-Angle Glaucoma have been identified in MYOC/TIGR gene, which encodes a 57kDa protein known as myocilin and in the gene NTF4 that codes a dimeric peptide (28kD). In this study we investigated the frequency of mutations in MYOC/TIGR and NTF4 genes in Kazakh population. The study was conducted involving 85 patients diagnosed with primary glaucoma and 100 individuals as a control group. The results of our research show that T353I, D208E in MYOC/TIGR gene and R206W polymorphisms in NTF4 gene among Kazakh population have no influence on the POAG progression while R76K SNP in MYOC/TIGR gene can be as a genetic factor which affects the development of POAG type of glaucoma.

Keywords: POAG, polymorphism, MYOC/TIGR, NTF4.

Introduction. Glaucoma is a neurodegenerative disease which is characterized by progressive damage to ganglion cells, optic nerve fibers, and visual field defects. It is one of the main reasons of irreversible blindness in the world. Primary Open-Angle Glaucoma (POAG) is a basic form of primary glaucoma. Glaucoma is a treatable disease if it is detected early, however, many patients get being diagnosed only after the loss of visual field, since glaucoma is typically asymptomatic at the early stages [1].

An estimated 66.5 million people were identified as having open-angle and angle-closure glaucoma by 2010 and it tends to reach 79.6 million by 2020. Binocular blindness in 2010 was observed in 8.4 million patients with glaucoma, and by 2020 it is going to rise to 11.2 million [2]. According to J. Goldberg's estimates, the number of glaucoma patients will reach 120 million by 2030 [3]. Since 2011, 24750 patients with glaucoma have been registered in Kazakhstan [4].

Primary open-angle glaucoma (POAG) is described distinctly as a multifactorial optic neuropathy that is progressive, and irreversible, with a characteristic acquired loss of optic nerve fibers. POAG is a chronic disease. It may be hereditary. Genetic predisposition is a distinctive feature of primary glaucoma and confirmed in 50% of cases. Currently, there are 4 causative genes and 70 candidate genes associated with the development of POAG [5]. The well-recognized genes associated with POAG include myocilin (MYOC/TIGR) [6, 7], optineurin (OPTN) [8] and neurotrophin-4 (NTF4) [9]. In the past 2 years, large scale genetic studies that have examined the blood samples of thousands of glaucoma patients have been instrumental in the discovery of more common genetic risk factors for POAG. For glaucoma, these genetic factors include changes in the DNA sequences or actual loss of DNA, and several different genes have been implicated [10]. How these genes cause or influence the likelihood of developing POAG is of major interest. The definition of a mutation in these genes is important for the diagnosis of glaucoma and genetic counseling of patients.

MYOC/TIGR gene located in chromosome 1q24.3 and expressed in many ocular tissues, including the trabecular meshwork. Therefore, the alternative name for this gene is TIGR (gene trabecular mesh-
work-included glucocorticoid response protein). The gene has 3 exons of size 604, 126, and 782 bp. 

*MYOC* is expressed as a 2.3 kb transcript and the translated product is predicted to contain 504 amino acids (58 kDa) [11]. Myocilin mutations, in general, are more strongly associated with POAG and JOAG than other forms of glaucoma [12, 13].

The next gene is neurotrophin (*NTF4*). Cytogenetic Location: 19q13.33, which is the long (q) arm of chromosome 19 at position 13.33. *NTF4* is translated as pre-pro-neurotrophin. The gene is organized in 2 exons and encodes a polypeptide of 210 amino acids. Neurotrophin protein is dimeric polypeptide with a molecular weight of 28 kDa and they are important regulators of neural survival, development, function, and plasticity. *NTF4* gene is expressed in most parts of the brain and in other tissues [14].

Mutations in the *MYOC/TIGR* and *NTF4* genes result in damage to actin fibers in the trabecular meshwork [15] and a decrease in neurotrophin signal [16]. Mutations in these genes are responsible for the development of glaucoma from 2% to 20%.

The main goal of this study is to investigate the polymorphism of *MYOC/TIGR* (rs772312298, rs2234926, D208E) and *NTF4* (rs121918427) genes in patients with glaucoma in population of Kazakhstan.

**Materials and methods.** The study was conducted involving 85 patients diagnosed with primary glaucoma. These materials were collected in the Kazakh Research Institute of Eye Diseases and in the Medical Centre Hospital of President’s Affairs Administration of the Republic of Kazakhstan. As a control group, people were selected who did not suffer from this disease and they were chosen depending on the age, gender and ethnic composition of patients with glaucoma. Genomic DNA was extracted from 200 μl of whole blood using a kit (ThermoFisher Scientific, USA). The concentration of the DNA molecule was determined using a DNA fluorometer (BioPhotometer plus, Eppendorf, Germany), and the quality was determined by agarose gel electrophoresis.

Genotyping of polymorphisms was carried out by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP). The volume of the reaction mixture for PCR is 20 μl: 50-100 ng of genomic DNA, 5 pmol of primer and Master Mix (ThermoFisher Scientific, USA). For the PCR thermal cycle, a touchdown annealing temperature of 62°C minus 0.2°C per cycle for 35 cycles was used in a thermal cycler (Mastercycler nexus, Eppendorf, Germany). Patients with POAG and members of the control group were also screened by restriction analysis. Restriction enzymes were mixed with each sample and incubated with their corresponding buffers overnight at 37°C (ThermoFisher Scientific, USA). Primer pairs and restriction enzymes are listed in table 1. DNA fragments were detected by electrophoresis on 2% agarose or 12% polyacrylamide gels.

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Primer pair (5’ → 3’)</th>
<th>Codon changes</th>
<th>Nucleotide changes</th>
<th>Restriction enzyme</th>
</tr>
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<tbody>
<tr>
<td>T353IR: T353F:</td>
<td>GCTACCCCTTAAAGTTCCATAC ATTGGCGACTGCTTAC</td>
<td>Thr&lt;sup&gt;353&lt;/sup&gt;lle</td>
<td>1058 (C→T)</td>
<td>HpyCH4III</td>
</tr>
<tr>
<td>R76KR: R76KF:</td>
<td>CTTCTGGACGGTGTGCTGCA CTGGTCCAAAGGTCATTTGTT</td>
<td>Arg&lt;sup&gt;76&lt;/sup&gt;Lys</td>
<td>227 (G→A)</td>
<td>BsmAI</td>
</tr>
<tr>
<td>D208ER: D208EF:</td>
<td>CATAGCTAATCTGGGCG CATTCAGACCTGCTCGACAA</td>
<td>Asp&lt;sup&gt;208&lt;/sup&gt;Glu</td>
<td>624 (C→G)</td>
<td>BsmAI</td>
</tr>
<tr>
<td>R206WR: R206WF:</td>
<td>CCGAGTCTGCGATTCTTCTGAGAAGAAGATTAC</td>
<td>Arg&lt;sup&gt;206&lt;/sup&gt;Trp</td>
<td>616 (C→T)</td>
<td>ApcI</td>
</tr>
</tbody>
</table>

When processing the PCR products with restriction enzymes, the following DNA fragments were obtained: Thr/Thr - 181, 101, 44, 30 bp.; Arg/Arg - 310 bp., Arg/Lys -310, 213, 97 bp, Lys/Lys - 213, 97 bp; Asp/Asp - 207, 126 bp; Arg/Arg-501,86, 71 bp.

**Results and discussion.** In order to observe *MYOC* and *NTF4* polymorphism by PCR-RFLP methods 85 patients suffering from glaucoma and 100 glaucoma-free individuals as a control group were included in genetic case-control studies. The genotype distribution based on SNPs are shown in figures 1 and 2.

Figure 1 – R76K SNP genotyping results by PCR-RFLP method

M – DNA Ladder 25 bp (*ThermoFisher Scientific, USA*),
1, 2, 3, 5 – GG genotype, 4, 6 – GA genotype, 7, 8, 9 – AA genotype.

Figure 2 – Results of R76K SNP polymorphism by PCR-RFLP method

The frequencies of R76K polymorphisms in glaucoma patients and healthy control group are tabulated in table 2.
It this study R76K SNP has been detected in 27 glaucoma patients (22-Arg/Lys, 5-Lys/Lys) and in 3 individuals (3-Arg/Lys) from the control group. 

R76K polymorphism is nucleotide change resulted in G being replaced by A (c.227G>A) in exon1 of MYOC/TIGR gene predicting amino acid change (substitution of Arg by Lys). The research carried out in Germany shows that R76K SNP was detected in 40 out of 112 glaucoma patients and in 3 patients there was identified Lys76Lys mutation at a polymorphic level [17].

According to the data, the variation T353I c.1058C>T in exon3 of MYOC gene can be one of the main reasons for developing POAG with increased intraocular pressure [15]. The reported findings on glaucoma genetics in Chinese Han population add to a growing body of evidence supporting that hypothesis. However, the genomic analyses of populations in Caucasus and Africa revealed that there is no genetic association of mutation T353I with glaucoma. Thus, genetic variations of MYOC gene can be varied among different ethnicity [16]. D208E mutations of MYOC/TIGR gene are causes of amino acid changes (Asp to Glu) due to the 624-cytosine nucleotide in exon2 of this gene is substituted by guanine. F. Mabuchi et al. reports that Asp208Glu mutations have been found in 4 hypertensive glaucoma patients and 3 POAG patients, at the same time it was revealed in 1 individual from the control group in Japan [18]. In addition, Japanese researchers investigated the distribution of Asp208Glu polymorphism among 99 glaucoma patients and their families also, in which Asp208Glu SNP has been indicated in one of the patients' mother and the researchers considered Asp208Glu polymorphism as an occasional neutral change with no effect on the gene's output [26]. Consequently, genetic variations of MYOC gene can be varied among different ethnicity.

The studies were carried out in China showed that R206W polymorphism in NTF4 gene is a rare example of mutation. This SNP was indicated only in one of the 174 patients [27]. The research held by Pasutto found that named SNP was indicated in 4 glaucoma patients out of 399 from the experimental group and in none of the controls [28]. Abundant studies lead us to conclude that R206W polymorphism is a very rare SNP. Indeed, as a result of our study R206W polymorphism has been detected in neither case belong to the test group nor the control group.

By the frequency of T353I, D208E and R206W polymorphisms in MYOC gene and in NTF4 respectively, there is no statistically significant difference between patients with POAG and individuals from control group. The available data point to the low frequency of above-mentioned polymorphisms. Our study has revealed that R76K is the most frequent polymorphism in Kazakhstan population. According to literary sources, the frequency of R76K SNP in Asia's population is higher than in Europe. The results of our research suggest that T353I, D208E and R206W polymorphisms in Kazakhstan population are the matter of neutral SNPs and could not be determined as a genetic reason of POAG progression, whereas R76K SNP has a strong association with POAG form of glaucoma.

REFERENCES


АННОТАЦИЯ. Қазақстанда біріншілік ашық бұрышты глюкозаңа қатысты глюкометрология саласындағы медициналық-элеуметтік марге не басым баяуыттардың бірі болып қалады. Глюкоза 21-50% жағдайда генетикалық негізделген, глюкомамен ауырытының наукалық ұранықтарына қашықтығы осы ауруларға дайындау 10 есе жоғары болатынығы анықталған. Біріншілік ашық бұрышты глюкозаңа ауырышының тұдырының мутациялары 57кДа мөлшерлі болғанын құрылымы MYOC/TIGR және 28 кДа димерлі полипептидің құрылымы NTF4 ғендерінде анықталған. Бұл құрметі Казахстан популяциясында MYOC/TIGR және NTF4 ғендеріндегі мутацияларын құралса жағдайға карағатырған. Зерттеу біріншілік ашық бұрышты глюкомамен ауырының 85 наукасы және баяуыт ретінде 100 сау адамдардан жиналған қауіпсіздік және қызметті құрылымды. Зерттеу қазіргісінде MYOC/TIGR ғеніндегі T3531, D208E және NTF4 ғеніндегі R206W мутациялары мен глюкозаңа ауырының құрылымы арқылы байланысты болмайды, ал MYOC/TIGR ғеніндегі R76K мутациясы ауруларға дайындауын есер ететіні дәліз анықталған.

Түнің сөзгері: БАБГ, полиморфизм, MYOC/TIGR, NTF4.

АНАЛИЗ ГЕНОВ МИОЦИЛИНА (MYOC) И НЕЙРОТОРОФИН 4 (NTF4) У БОЛЬНЫХ ГЛУКОМОЙ В КАЗАХСТАНСКОЙ ПОПУЛЯЦИИ

Аннотация. Первая открытая глюкома, являющаяся наиболее распространенной формой глюкома, имеет большое социально-медицинское значение и является основным направлением научных исследований в области глюкомологии. В 21-50% случаев заболевание обусловливается генетически, а у потомков людей, болеющих глюкомой, риск этой патологии в 10 раз выше. Мутации, вызывающие первичную открытую глюкомой, были идентифицированы в гене MYOC/TIGR, который кодирует белок, известный как миоцилин (57 кДа), и в гене NTF4, который кодирует димерный пептид (28кД). В данном исследовании мы изучали частоту мутаций в генах MYOC/TIGR и NTF4 среди населения Казахстана. Исследование проводилось с участием 85 пациентов с первичной глюкомой и 100 человек в качестве контрольной группы. Результаты наших исследований показывают, что полиморфизм T3531, D208E в гене MYOC/TIGR и R206W в гене NTF4 среди населения Казахстана не влияют на прогрессию ПОУГ, тогда как R76K SNP в генах MYOC/TIGR может быть генетическим фактором, который влияет на развитие глюкомы типа ПОУГ.

Ключевые слова: ПОУГ, полиморфизм, MYOC/TIGR, NTF4.

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