Effect of TXNRD2 rs35934224, FOXC1 rs2745599 and rs984253 genetic polymorphisms combinations on the development of primary open-angle glaucoma and their degree of association with the disease

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Primary open-angle glaucoma (POAG) is a complex disease caused by numerous genetic and environmental factors, as well as their interaction. In recent studies, the effect of genetic polymorphisms combinations and non-equilibrium linkage of allele genes related to the development of POAG has been proved. The aim of the study was to determine the effect of TXNRD2 rs35934224, FOXC1 rs2745599 and rs984253 genetic polymorphisms combinations on the development of primary open-angle glaucoma and their degree of association with the disease. The study included 93 patients (185 eyes) with POAG stage I-IV and 89 volunteers (178 eyes) control subjects without any types of glaucoma. The patients were divided into four groups according to the degree of perimetric changes (Nesterov A. P., 2008). All patients performed visometry, computer perimetry, tonometry, biomicroscopy, ophthalmoscopy, gonioscopy, keratometry, optical coherent tomography of the optic nerve. Analysis of the TXNRD2 rs35934224, FOXC1 rs2745599 and rs984253 genetic polymorphism with POAG was performed in real time using a polymerase chain reaction (PCR) in Gene Amp® PCR System 7500 (Applied Biosystems, USA) automatic amplifier. In the first stage of the study, the genomic DNA from whole venous blood was isolated using the standard reagents PureLink® Genomic DNA Kit for purification of genomic DNA, manufactured by INVITROGEN (USA). The analysis of polymorphism was carried out using unified test systems of TaqMan Mutation Detection Assays Life-Technology (USA). It was determined that the association with POAG had the genotype C/T*A/A*T/A as by comparing control with all patients, and by stratification - with the 1st, 2nd and 3rd groups of patients. The obtained results showed the evidentiary effect of this genotype combinations on the appearance of POAG, and on its progression by the stages of perimetric changes. The risk of the occurrence of POAG in carriers of genotypes C/T*A/A*T/A was increased by 2.8 times (p<0.001). In this combination, the two polymorphisms had heterozygous genotypes (rs35934224 - C/T, rs984253 - T/A), and the genotype rs2745599 - a mutant homozygote A/A. A combination of genotypes C/C*G/A*T/A was also important for the progression of the disease till stage II, which increased the risk of development of the POAG stage II by 2.9 times (p<0.01) compared to control. The risk of occurrence of the POAG in general and development of stage IV increased the presence of combinations of three minor genotypes T/T*A/A*A/A, which was encountered only in patients with POAG (in stage II - f = 0.025, in the third stage - f = 0.036, and in IV - f = 0.071). In our opinion, it confirmed the proposed working hypothesis of the study and showed that the more genotype combinations have the mutant alleles, the stronger this genotype affects the development of POAG.

Keywords: primary open-angle glaucoma, chromosome, genotype, polymorphism, TXNRD2 gene, FOXC1 gene, allele, heterozygote.
genetics have shown that POAG can be caused by numerous gene mutations in various chromosomal loci. A study performed by Cong G. et al. estimated that inherited and familial POAG cases may account for approximately 72% of all POAG cases [6, 7, 8, 22, 27].

Another gene for testing as the genetic marker of the primary open-angle glaucoma (POAG) has become the developmental gene: forkhead box C1 (FOXC1) [1, 21]. This gene plays an important role in the normal morphogenesis of the anterior segment of the eye and is involved in the regulation of intraocular pressure and the function of the trabecular mesh [21]. The FOXC1 gene (previously FKHL7) is one of the six known genes of glaucoma [28]. Although FOXC1 expression has not been studied in adults yet, it is possible that prolonging the expression of an abnormal gene product (from age-related, sub-clinical mutations) throughout life, or altering the expression level of FOXC1 may affect the normal function of the trabecular mesh, thereby leading to an increased risk for the development of POAG through difficulty in drainage and increased intraocular pressure [26].

Another gene that has attracted our attention is the TXNRD2 gene encoding the mitochondrial protein, thioredoxin reductase 2, which is required for mitochondrial redox homeostasis. Thioredoxin 2 reduces the damaging effect of active forms of oxygen, which are formed as a result of oxidative phosphorylation [4]. And the decrease in the number of active forms of oxygen by activating the expression of the TXNRD2 gene prevents mitochondrial dysfunction and apoptosis of ganglion cells in POAG [1, 2].

In recent proceedings devoted to genetic research in the POAG [1, 21], the effects of combinations of polymorphic genotypes and non-equilibrium linkage of allele genes related to the development of POAG were considered. This approach is a very promising area of research, since a particular patient has a certain common genotype consisting of the specific polymorphic genotypes combinations.

That is why, in our opinion, when conducting studies of several polymorphic genes it is advisable to consider the association of genotype combinations with the development of the disease.

The aim of the study was to determine the effect of TXNRD2 rs35934224, FOXC1 rs2745599 and rs984253 genetic polymorphisms combinations on the development of primary open-angle glaucoma and their degree of association with the disease.

Materials and methods

The research was performed at the Department of Eye Diseases of the National Pirogov Memorial Medical University and the department of ophthalmology of Vinnitsa Regional Clinical Hospital Named After N. I. Pirogov. The examination of patients and the diagnosis of POAG was carried out in accordance with the classification of A. P. Nesterov perimetric changes in the stages of glaucoma [17].

The study included 93 patients (185 eyes) with POAG stage I-IV and 89 volunteers (178 eyes) control subjects without any types of glaucoma. The patients were divided into four groups according to the degree of perimetric changes [17]. All patients performed visometry, computer perimetry, tonometry, biomicroscopy, ophthalmoscopy, gonioscopy, keratopahymetry, optical coherent tomography of the optic nerve.

All stages of molecular genetic research were carried out at the Research Institute of Experimental and Clinical Medicine of the O. O. Bogomolets National Medical University (chief - m.d., professor Natrus L.V.). Analysis of the TXNRD2 rs35934224, FOXC1 rs2745599 and rs984253 genetic polymorphism with POAG was performed in real time using a polymerase chain reaction (PCR) in Gene Amp® PCR System 7500 (Applied Biosystems, USA) automatic amplifier. In the first stage of the study, the genomic DNA from whole venous blood was isolated using the standard reagents PureLink® Genomic DNA Kit for purification of genomic DNA, manufactured by INVITROGEN (USA). The analysis of polymorphism was carried out using unified test systems of TaqMan Mutation Detection Assays Life-Technology (USA). For statistical analysis of the results, MedStat and MedCalc v.15.1 (MedCalc Software bvba) were used. Association of genotypes and alleles with the disease were determined by the odds ratio (OR); The limits of 95% of the credible interval (Cl) were calculated by the method of J. Neyman. The differences were statistically significant at p<0.05. To test the probability of differences between groups, χ² was used and Fischer's exact criterion was used.

Results

The association with the disease has been confirmed only for genotype combinations C/C*A/A*T/A. It was shown that this genotype was found in the control group with a frequency of f = 0.101, and with POAG - with frequency f = 0.238, that is, 2.4 times more often (p<0.001).

The genotype combinations C/C*A/A*T/A increased the chances of development of the POAG compared to the control by 2.8 times (OR = 2.775; 95% CI = 1.532-5.021). Also, the genotype combinations of T/T*A/A*A*A increased the risk of development POAG (p<0.05), since such genotype wasn't detected in the control group. It should be noted that this genotype is a combination of all three mutant homozygotes. This fact confirmed the negative effect of the mutant alleles of all three polymorphisms on the occurrence of POAG.

According to the Table 1, four genotype combinations C/C*A/A*T/T, C/C/G/G*A/T/T, C/C/G/G*T/T and C/T/G/A*T/T showed a strong protective effect on POAG and substantially reduced the chances of development POAG.

The maximum power of connection was determined to genotype combinations C/C/G/G*T/T, which reduced the chances of development POAG by 7.7 times (OR = 0.132; 95% CI = 0.044-0.338). The genotype combinations C/T/G/A*T/T reduced the chances by 6.7 times (OR = 0.157; 95% CI = 0.035-0.697) and the genotype combinations C/C/A*A/T/T and C/C/G/A*T/T - 3.8 times for both cases (OR = 0.263; 95% CI = 0.101-0.685).
The disease (OR > 0) according to the tables 1 and 2. Comparisons was found, as well as the associative relation with significance of the differences at level p < 0.05 in the groups of control group and in patients with POAG when stratified in groups.

**Table 1.** Influence of genotype combinations on the development of POAG and their degree of association with the disease.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>POAG</th>
<th>Control</th>
<th>χ²</th>
<th>P</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>C/C<em>A/A</em>T/A</td>
<td>0.242</td>
<td>0.103</td>
<td>11.03</td>
<td>&lt;0.001</td>
<td>2.775</td>
<td>1.532-5.021</td>
</tr>
<tr>
<td>C/C<em>A/A</em>T/T</td>
<td>0.033</td>
<td>0.111</td>
<td>7.552</td>
<td>&lt;0.01</td>
<td>0.263</td>
<td>0.101-0.685</td>
</tr>
<tr>
<td>C/C<em>G/G</em>T/T</td>
<td>0.033</td>
<td>0.111</td>
<td>7.552</td>
<td>&lt;0.01</td>
<td>0.263</td>
<td>0.101-0.685</td>
</tr>
<tr>
<td>C/T<em>G/A</em>T/T</td>
<td>0.024</td>
<td>0.152</td>
<td>16.93</td>
<td>&lt;0.001</td>
<td>0.132</td>
<td>0.044-0.385</td>
</tr>
<tr>
<td>T/T<em>A</em>A*A</td>
<td>0.015</td>
<td>0.073</td>
<td>6.392</td>
<td>&lt;0.05</td>
<td>0.157</td>
<td>0.035-0.697</td>
</tr>
<tr>
<td>T/T<em>A</em>A*A</td>
<td>0.031</td>
<td>0.001</td>
<td>4.044</td>
<td>&lt;0.05</td>
<td>max</td>
<td>N/A-max</td>
</tr>
</tbody>
</table>

**Notes:** χ² - Pearson's χ-square criterion; p - statistical significance; OR - odds ratio; 95% CI - 95% credible interval for OR.

**In this regard,** at the next stage, the character of the distribution (Fig. 1) and the connection (Table 2) of the genotype combinations with POAG in stratification by groups was determined.

Among the risk genotypes, association with the disease has been confirmed only for genotype combinations C/C*A/A*T/A. It was shown above that this genotype was found in the control group with a frequency f = 0.101, and in POAG - with frequency f = 0.238, which is 2.4 times more often (p<0.001).

**Henceforward,** the nature of the connection between the genotype combinations with the disease when stratified by groups was calculated (Table 2).

In the 1st group, as in all patients with POAG, the association with the disease was determined only for genotype combinations C/C*A/A*T/A (χ² = 18.86; p<0.001), which in 8, 1 times increased the chances of development POAG (OR = 0.086; 95% CI = 3.025-21.65).

In the 2nd group, the genotype combinations C/C*A/A*T/A (χ² = 5.852; p<0.05) increased the chances of development POAG by 2.5 times (OR = 2.541; 95% CI = 1.244-5.197) as well as the genotype combinations C/C*G/G*T/A (χ² = 6.564; p<0.01), which increased the chances by 2.9 times (OR = 2.885; 95% CI = 1.336-6.243).

Two other genotype combinations, which in the 2nd group had a statistically significant association with POAG, reduced the chances of its development: C/C*G/G*T/T (χ² = 7.295; p<0.01) in 6.7 times (OR = 0.154; 95% CI = 0.033-0.642) and C/C*G/G*T/T (χ² = 4.434; p<0.05) - 5.0 times (OR = 0.203; 95% CI = 0.052-0.886).

In the 3rd group, reasonable differences were found for the risk genotype combinations C/T*A*A*T/A (χ² = 6.933; p<0.01), which increased the chances of development POAG by 3.7 times (OR = 1.464-9.524) and for the protective genotype combinations C/C*G/G*T/T (χ² = 3.884; p<0.05), which reduced the chances of development POAG by 4.5 times (OR = 0.223; 95% CI = 0.051-0.965).

In the 4th group, the potentiating action of the combination of three mutant genotypes T/T*A*A*A (χ² = 6.483; p<0.05) was shown, which increased the chances of development POAG.

**Discussion**

Although increased intraocular pressure (IOP) is a key risk factor for primary open-angle glaucoma, the fact that about one third of European-born patients with glaucoma have a normal intraocular pressure (normotensive glaucoma) suggests that other factors may also affect the propensity to degeneration of optic nerve [9, 20].

There are increasing evidences of mitochondrial dysfunction in the susceptibility of the optic nerve to glaucoma [1, 5, 12, 14, 19, 23]. The retinal ganglion cell axons don't have a myelin sheath, so that sufficient amount of adenosine triphosphate (ATP) to maintain the potency of action in the absence of myelin is provided by a sufficient number of mitochondria. The optic nerve is a site that is particularly...
susceptible to mitochondrial dysfunction [3, 13].

Genetic studies have shown that nuclear genes encoding mitochondrial proteins may contribute to the risk of COPD [10]. Recently, a large association of genomic studies revealed a significant association of POAG with single nucleotide polymorphism (SNPs) in the genomic region of TXNRD2, a nuclear-coded mitochondrial protein [1].

In previous studies, we determined the association of the rs35934224 polymorphism of the TXNRD2 gene with primary open-angle glaucoma. It was found that the polymorphism rs35934224 had differences in the distribution of genotypes and alleles between patients with POAG and control group. Moreover, the shift of alleles and genotypes toward minor causes increased severity of the pathological process. But the stratification of the perimeter changes in the POAG showed that the association with the disease was significant at stages III and IV. Moreover, the chances of development of the III stage of POAG were significantly higher in comparison with the I stage in carriers of C/T and T/T genotypes and allele T. In the same plan, the protective effect of the ancestral genotype C/C was also strengthened [15].

FOXC1 is a gene that regulates the development of the anterior segment of the eye and is known to play a role in several autosomal dominant eye defects associated with increased risk of glaucoma, including the Axenfeld-Rieger anomaly, iris hypoplasia and Rieger syndrome [16, 18].

Foxc1 is expressed in the embryonic trabecular meshwork (TM) [25], the continuation of the expression of an abnormal gene product (from age-related, sub-clinical mutations) throughout life, or altered expression levels of FOXC1 may affect the normal function of the trabecular meshwork, thereby leading to an increased risk of glaucoma due to increased intraocular pressure. This concept is confirmed by the fact that glaucoma associated with mutations in the genes of glaucoma development may occur at any time from birth to adulthood, and in some cases more than 70 years [24]. Moreover, in some patients with glaucoma due to FOXC1 mutations, the anomalies of the anterior segment can be very thin and easily passed through a clinical examination [11, 24], which is more consistent with POAG.

The connection of the polymorphism of the FOXC1 gene to the development of POAG has been studied in individual studies. Thus, in a study by British scientists [21] four polymorphisms were studied: rs2235715, rs2569889, rs2235716 and rs984253. In this case, all these polymorphisms do not cling to the coding region of the gene, and only the latter is located in the non-coding intron of the FOXC1 gene (16 13 294). We conducted a study of the association of polymorphisms rs984253 and rs2745599 of the FOXC1 gene with POAG. In which it was discovered that the distribution of genotypes and alleles had a relationship with POAG, but polymorphism rs984253 had no relation to its progression in the stages of the pathological process. While the polymorphism rs2745599 of the FOXC1 gene was related to the formation of the POAG of the most severe (IV) degree, and the risk of rapid progression of the disease was greater in the carriers of the minor allele A.

In this study, we determined the effects of the combinations of genotypes of polymorphisms on the development of POAG and the degree of their association with the disease. We found that the association with the disease had the genotype C/T/A/A*T/A as a comparison of control with all patients, and with stratification - from the 1st, 2nd and 3rd groups of patients. In our opinion, this convincingly indicated the evidentiary effect of this combination of genotypes and on the occurrence of POAG, and on its progression in the stages of perimetric changes. The risk of occurrence of POAGs in carriers of C/T/A/A*T/A genotypes was increased by 2.8 times (p<0.001).

Interestingly, in this combination, the two polymorphisms had heterozygous genotypes (rs35934224 - C/T, rs984253 - T/A), and the genotype rs2745599 was a mutant homozygote A/A.

A combination of C/C*G/A*T/A genotypes was also important for the progression of the disease to stage II, which increased the risk of developing the II stage of POAG by 2.9 times (p<0.01) compared to control.

The risk of both general POAG and stage IV development increased the presence of combinations of three minor T/T*A/A*A/A genotypes that were found only in patients with POAG (at stage II if f = 0.025, in stage III - f = 0.036, and for IV - f = 0.071). In our opinion, this testified in favor of the proposed working hypothesis of the study and confirmed that the more combined the genotypes of the mutant alleles, the stronger this genotype affects the development of POAG.

Conclusions

1. The association with the disease had the genotype C/T/A/A*A/T/A as a comparison of control with all patients, and with stratification - with the 1st, 2nd and 3rd groups of patients. The risk of the occurrence of POAG in carriers of C/T/A/A*A/A genotypes was increased by 2.8 times (OR = 2.775; 95% CI = 1.532-5.021). Also, the combination of minor T/T*A/A*A/A genotypes made a significant increase in the risk of development of POAG (p<0.05), since no such genotype was detected in the control group.

2. For the progression of the disease to the II stage, the combination of C/C*G/A*T/A genotypes was also important, which increased the risk of development of the II stage of POAG in 2.9 times (p<0.01) as compared with the control.

3. The probable protective effect on the POAG was determined to combine the genotypes C/C*G/G*T/T, which reduced the chances of development of the POAG by 7.7 times (OR = 0.132; 95% CI = 0.044-0.383). The combination of genotypes C/T/G/A*T/T reduced the chances by 6.7 times (OR = 0.157; 95% CI = 0.035-0.697) and the combination of genotypes C/C*A/A*T/T and C/C/G/A*T/T - 3.8 times for both cases (OR = 0.263; 95% CI = 0.101-0.685).
References


ВЛИЯНИЕ СОЕДИНЕНИЙ ГЕНОТИПОВ ПОЛИМОРФИЗМОВ RS35934224 ГЕНА TXNDR2, RS2745599 И RS984253 ГЕНА FOXC1 НА РАЗВИТИЕ ПЕРВИЧНОЙ ОТКРЫТОГОГУЛЬНОЙ ГЛАУКОМЫ И СТЕПЕНЬ ИХ АССОЦИАЦИЙ С ЗАБОЛЕВАНИЕМ

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Первичная открытоглазой глаукома (ПОУГ) - это сложное заболевание, вызванное многочисленными генетическими и экологическими факторами, и также их взаимодействиями. В последних научных исследованиях было доказано влияние соединений полиморфных генотипов и неравновесное сцепление аллелей генов, имеющих отношение к развитию ПОУГ. Цель исследования - определение влияния соединений генотипов полиморфизмов rs35934224 гена TXNDR2, rs2745599 и rs984253 гена FOXC1 на развитие ПОУГ и степень их ассоциаций с заболеванием. Проведено исследование 93 больных (185 глаз) с ПОУГ I-IV стадий и 90 здоровых лиц (178 глаз), у которых не было установлено каких-либо глазных, состоявших контрольную группу. Больные были разделены на 4 группы согласно степени периметрических изменений (Nesterov A. P., 2008). Всем больным выполнена визометрия, компьютерная периметрия, тонометрия, биомикроскопия, офтальмоскопия, генотипика, оптика наружного глазного нерва. Анализ полиморфизмов rs35934224 гена TXNDR2, rs2745599 и rs984253 гена FOXC1 проведено методом полимеразной цепной реакции в реальном времени в автоматическом амплификаторе Gene Amp® PCR System 7500 (Applied Biosystems, США). На первом этапе исследования проводили выделение геномной ДНК из цельной венозной крови с использованием стандартных реактивов PureLink® Genomic DNA Kit For purification of genomic DNA, выробник INVITROGEN (США). Анализ полиморфизма rs35934224 zдійснено з використанням уніфікованих тест-систем TaqMan Mutation Detection Assays Life-Technology (Уніфіковані тест-системы TaqMan Mutation Detection Assays Life-Technology). Проведено дослідження проводили виконання геномної ДНК з цельної венозної крові з використанням стандартних реактивів з уніфікованими тест-системами TaqMan Mutation Detection Assays Life-Technology (Уніфіковані тест-системы TaqMan Mutation Detection Assays Life-Technology). Здійснено з використанням уніфікованих тест-систем TaqMan Mutation Detection Assays Life-Technology. Здійснено з використанням уніфікованих тест-систем TaqMan Mutation Detection Assays Life-Technology. Анализ полиморфизма rs35934224 - f = 0,036, что ассоциировало с ПОУГ имел генотип C/T*A/A*T/A, яке зустрічалось тільки у хворих на ПВКГ (f=0,025, при III стадії - f=0,036, а при IV - f=0,071). На наш погляд, це сприяло виникнення високому ризику розвитку PPOU в у 2,8 раз (p<0,001). У цьому сполученні два поліморфізми мали гетерозиготні генотипи (rs35934224 - C/T, rs984253 - T/A), а генотип rs2745599 - мутантну гомозиготу A/A. Для прогресування захворювання до II стадії мало значення також сполучення генотипів C/C*Г/A*T/A, яке збільшує ризик розвитку II стадії ПОУГ в 2,9 раз (p<0,01) у порівнянні з контролем. Ризик як зазначено ПОУГ, так і розвитку IV стадії підвищувала наявність сполучення трьох мінорних генотипів T/А*А/А, яке зустрічалось тільки у хворих на ПВГ (при II стадії f=0,025, при III стадії - f=0,036, а при IV - f=0,071). На наш погляд, це сприяло виникнення високому ризику розвитку ПОУГ у носіїв сполучення генотипів мутантних аллелей, тим сильніше такий генотип впливає на розвиток ПОУГ.

Ключевые слова: первична открытоглазальная глаукома, хромосома, генотип, полиморфизм, ген TNXDR2, ген FOXC1, аллергия, гетерозигота.