

## GENETIC BACKGROUND OF PRIMARY CONGENITAL GLAUCOMA

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**S u m m a r y.** Glaucoma is a neurodegenerative disease causing the gradual loss of retinal ganglion cells and optic nerve damage, which results in blindness. Glaucoma aethiology is unknown and the increased intraocular pressure is the only modifiable risk factor in the development of the disease. Congenital glaucoma and juvenile-onset open angle glaucoma are associated with the abnormal development of the anterior segment of the eye, especially the trabecular meshwork, which is connected with aqueous humour outflow pathways and high intraocular pressure. Researches have often pointed to the role of specific gene defects that contribute to the pathogenesis of the glaucoma. The identification of genes will help us to define the underlying pathophysiology, moreover can lead us to develop the new diagnostic test and provide information on better treatment.

**Key words:** congenital glaucoma, blindness, *CYP1B1* gene, *LTBP2* gene

### GENETIC BACKGROUND OF PRIMARY CONGENITAL GLAUCOMA

Primary congenital glaucoma is a separate nosological unit characterised by the appearance of optic neuropathy in early life in newborn babies or children between 2 and 16. Primary congenital glaucoma is believed to be caused by the abnormal eye structure, namely the dysgenesis of the iridocorneal angle, in particular trabecular meshwork, through which the aqueous humour from the anterior chamber of the eyeball flows to the scleral venous sinus, proceeding through aqueous veins to the circulatory system. The improper drainage of aqueous humour leads to its accumulation in the eyeball raising intraocular pressure, which can lead to the eye ischaemia, death

of retinal ganglion cells, atrophy of the optic nerve, which consists of axons from the retinal ganglion, leading to blindness in early life.

Primary congenital glaucoma occurs rarely, approximately in 1:10000 births. Boys account for approximately 65% of cases. It can be divided into three main categories:

- a) Primary newborn glaucoma is characterised by increased intraocular pressure during foetal development (40% of cases)
- b) Primary infantile glaucoma is a type of glaucoma recognised in the two first years of life (55% of cases)
- c) Late-recognised primary infantile glaucoma is recognised between 3 and 16 years of age.

Genetic predispositions seem to be among the factors causing glaucoma. In about 10% of cases glaucoma is inherited as an autosomal recessive condition, especially among closely related people. In the course of research three loci for PCG on corresponding chromosomes have been found: *GLC3A* (2p21), *GLC3B* (1p36), *GLC3C* (14q24.3-q31.1). However, the biggest significance is attributed to *GLC3A* located on chromosome 2. The mutations observed in this locus influence the Cytochrome P450 1B1 (*CYP1B1*) gene and appear in approximately 50% of cases [2].

The *CYP1B1* gene is located on chromosome 2 and contains three exons and two introns. The coding region of *CYP1B1* gene starts at the 5' end of the second exon and ends within the third exon. It codes a protein product of 543 amino acids, that appears in human eye tissues (anterior segment

of the eyeball) and other tissues of the body. It functions as a monomeric mixed-function oxidase, whose substrates play an important role in the eyeball development, however, it is not clear what role that is. Its involvement in various pathways, also involving steroids and retinoids, has been described in multiple works. It has been proven to participate in 17 $\beta$ -estradiol metabolism [6, 7, 10]. It catalyses some of the oxidising reactions, some of which are biosynthetic reactions, that create hormones or components participating in complicated metabolic pathways in the majority of living organisms including multiple xenobiotics, steroids and vitamins, for instance, it metabolises vitamin A in two steps to all-trans retinal and all-trans-retinoic acid as its products. The latter is a potent morphogen regulating in the utero development of tissue growth and differentiation. The *CYP1B1* gene is involved in metabolism of endogenous and exogenous substances, which participate in the early stages of ocular tissue differentiation [5, 12, 13].

The cytochromes membrane such as CYP1B1 have molecular structure containing a transmembrane domain located at the N-terminal end of the molecule, that is proline-rich regions which permit flexibility between the membrane-spanning domain and the cytoplasmic portion penetrated by protein molecules. The cytochrome P450 family contains a basic set of structures responsible for the heme-binding region of these molecules. The heme-binding region is essential for the normal functioning of every P450 molecule. Like any protein, the cytochrome P450 functions as the classical enzyme molecule [4, 12]. The mutations in recessive phenotypes lead to the production of incorrect enzymes because the subjects having the normal heterozygous allele are capable of compensating for the mutant allele. Mutations in the *CYP1B1* gene interfere with the integrity of the CYP1B1 coded protein, its ability to adopt normal conformation and to bind heme, for example induced mutations in the hinge region interfere with the heme-binding properties of the cytochrome P450 molecules [13].

The studies of pathogenic sequence variants of *CYP1B1* in different populations have contributed to the better understanding of the molecular pathogenesis of congenital glaucoma. The changes in metabolism of the above mentioned pathways caused by the mutations in *CYP1B1* gene and the lack of normal substrates may considerably influence the mechanism of aqueous flow, and

thus contribute to the development of congenital glaucoma. They also helped to show a link between the structure and function of different *CYP1B1* gene mutations in the cases of people with recognised mutations and developing glaucoma, especially the relatives of patients with PCG. This is of great clinical significance. The monitoring of such families seems justified, as it may lead to decrease in congenital glaucoma in familial cases. Any research in this area leads not only to the better understanding of pathogenic mutations in different populations, but also to the development of simple diagnostic markers for detection and analysis of such cases. This may lead to the development of novel therapeutics in congenital glaucoma management [8, 13].

Initially, the genetic research was conducted mainly on mice. Incomplete penetrance and diverse gene expression suggested an additional genetic modifier. It is believed that when the genetic modifier interacts with *CYP1B1* gene it increases the probability of glaucoma and intensifies visual disturbances. The modifier genes have been determined based on research in which the gene coding tyrosinase (*TYR*) was recognised as the modifying gene increasing the probability of angle dysgenesis [9]. CYP1B1 protein caused more malformations of the angle in the mice deficient in the *TYR* gene than in the mice with proper levels of the *TYR* gene, but deficient in CYP1B1. L-Dopa, a molecule crucial for the development of vision, is the product of tyrosinase [6]. Therefore, other genes that influence L-DOPA or L-DOPA catecholamine metabolites may also modify the glaucoma phenotype. These results indicate high usefulness of mice for determining the multifactorial genetic effects under strictly controlled conditions (both genetic and environmental) and suggest, that the mutations in these genes may cause glaucoma in humans as well [6]. The tests performed on people proved that CYP1B1 mutations may disturb not only the above mentioned pathways, but also the proper development of the eyeball, especially trabecular meshwork or Schlemm's canal, which may result in congenital glaucoma. The studies of different groups of patients e.g. from Saudi Arabia, Turkey, Slovakia, Romania, the USA or Brazil provided comparable results, which led to similar conclusions. Research to date has shown approximately 44 different mutations in the whole *CYP1B1* gene coding region and the diversity of mutations varies depending on population. Although genetic heterogeneity has been reported

in PCG, homogeneity in phenotype as well as genotype (E387K) has been reported in the Slovak and Gypsy populations, and common haplotypes (G61E, D374N and R469W) have been associated in the Saudi Arabian population [8]. It appears, that CYP1B1 plays a crucial role in primary congenital glaucoma development, especially in its most severe cases, and its product - cytochrome P450, dependant on arachidonic acid (product of metabolism) blocks Na, K ATPase in cornea, which may influence the transparency of cornea and aqueous humour production [1].

The detection of mutations in *CYP1B1* gene in the conducted tests have proven that *CYP1B1* gene mutations are responsible for primary congenital glaucoma among many children [3]. The tests were conducted on the patients divided into three categories based on the histological phenotype of the angle: 1 – severe goniodysgenesis characterised by the agenesis of Schlemm's canal, 2 – moderate goniodysgenesis and 3 – mild goniodysgenesis with the deposition of a mucopolysaccharide material in the juxtacanalicular tissue. CYP1B1 mutations in the case of both moderate and severe dysgenesis correlated with disease severity. The majority of cases in the test groups manifested heterozygous CYP1B1 mutations, it may be therefore concluded that specific CYP1B1 mutations may be connected with severe and moderate visual disturbances [5].

The *LTBP2* gene is yet another heavily investigated gene. Although this gene is not well-researched, the assumption is that mutations in *LTBP2* gene are greatly responsible for the primary congenital glaucoma [2, 7]. *LTBP2* gene is one of the largest units of the latent transforming growth factor (TGF) - beta binding protein family, which is composed of extracellular matrix proteins with the multidomain structure. The protein constructed of 1821 amino acid sequences, is encoded by 35 exons - a part of the *LTBP2* gene. The analysis indicates that 20 domains are responsible for encoding the epidermal growth factor (EGF), a 4 transforming growth factor beta binding protein (TB), each of the remaining modules contains 8 cysteine residues and an amino-terminal signal peptide [2]. Due to its similarities to fibrillins in terms of structure, it appears that it may play a role of a structural element, (as a component of microfibrils) or/and in the process of cell adhesion, as a docking molecule for elastic fibre assembly. The presence of LTBP2 protein in the anterior sector of the eyeball, at the ciliary body, may suggest its particular engagement in structure

precesses. Furthermore, the tests have proven that LTBP2 is a vital factor for the development of the anterior chamber of the eye, and ciliary body together with trabecular meshwork in particular. Nevertheless, neither precise mechanisms of *LTBP2* gene mutations nor the effects of these mutations, which may result in PCG, are known. The analysis of LTBP2 distribution in the anterior segment of the eyeball was primarily conducted on mice and cows [2], and only recently on human patients [6]. The observations in question revealed that defective LTBP2 may influence the structure of the organs elastic tissue. Moreover, cause changes in the structural support of surrounding tissues. This defect is predominantly connected with Schlemm's canal whose elasticity and structure greatly influence proper aqueous outflow, and the defect of which is considered to be one of the key factors leading to glaucoma [2, 7].

Primary congenital glaucoma is a separate nosological unit, which may also occur in complex developmental disorders in the anterior segment of the eye, and is referred to then as secondary congenital glaucoma. The nosological units which can be distinguished here are the following:

- a) Axenfeld-Rieger syndrome
- b) Aniridia

Axenfeld-Rieger syndrome is a congenital disorder associated with anterior segment dysgenesis. The syndrome comprises three nosological units, where the genetic background and defects are determined by different genetic abnormalities, which makes them separate units on the genetic level. The following conditions can be distinguished:

- Axenfeld anomaly – the main source of disorders may be found in the angle dysgenesis, where peripheral iris tissue strands are attached to a prominent anteriorly displaced Schwalbe's line.
- Rieger anomaly – is an autosomal dominant-inherited disorder characterised by prominent changes. These may include: Schwalbe's line displacement into the anterior chamber, iris hypoplasia, the eye's pupil displacement (corectopia) and the iris full-thickness colobomas (pseudopolycoria). In the case of this condition, glaucoma is quite frequent as it develops in 50% of patients.
- The Rieger's syndrome, apart from the aforementioned anomalies of the anterior segment of the eyeball, includes the following: the decreased number of teeth (hypodontia), the decreased size of teeth (microdontia), the facial anomalies (a broad

flat nasal bridge, a lateral displacement of the medial canthus and an increased distance between the bony orbits) [1, 3].

Axenfeld-Rieger syndrome has been thoroughly examined when it comes to genetic disorders. It has been linked to five chromosomal loci (4q25, 6p25, 11p13, 13q14, 16q24) and mutations have been identified in three transcription factor genes, two of which - *PITX2* gene and *FOXC1* gene map to chromosomes 4q25 and 6p25, respectively – are the most frequently affected. Only one case of mutation has been observed in *PAX6* gene, which maps to chromosome 11p13. The *FOXC1* gene is located on 6p25 and has a single exon, responsible for coding for the protein of 553 amino acids, which most probably plays a role of FKHL7 transcription factor. The *FOXC1* protein is found in different ocular and non-ocular tissues, e.g. in periocular mesenchyme cells which build eye structures such as the iris, cornea and trabecular meshwork [1, 3]. In mice two populations were distinguished: the *FOXC1* gene null and the heterozygous *FOXC1* gene. Both are characterised by anterior segment anomalies, predominantly including small or absent Schlemm's canal, the trabecular meshwork malformation, the iris underdevelopment, the displaced Schwalbe's line, such as in primary congenital glaucoma or anterior segment dysgenesis (ASD) [9]. *FOXC1* mutations are believed to be closely connected with anterior segment dysgenesis (ASD), such as iridogoniodysgenesis, Axenfeld-Rieger syndrome (ARS) or Peter's anomaly. The aforementioned congenital anomalies of the eyeball often coexist with primary newborn glaucoma or primary infantile glaucoma (50% - 75% of cases), which may suggest that transcription factor *FOXC1* plays an important role in these nosological unit as well [3, 6, 11].

Despite these indications, research on *FOXC1* gene has been insufficient to associate its function, or the lack of it, with classic PCG cases. There is, however, possibility that *FOXC1* gene contributes to PCG pathogenesis predominantly through interaction with another transcription factor on particular stages of a biochemical pathway and their mutation-based disturbances [1]. The *PITX2* gene coded transcription factor seems to plays this role. This gene remains in direct interaction with *FOXC1* and regulates its activity. In the cells, which express both *FOXC1* and *PITX2* proteins, *PITX2* products are more active and the target gene, *FOXC1*, is inhibited by *FOXC1*–*PITX2* protein

complexes. The loss of *PITX2* gene function due to mutations, or the reduced expression of *PITX2* gene, greatly influences the *FOXC1* gene and results in its inappropriate activation [11]. Tight correlation between *FOXC1* and *PITX2* appears crucial for normal development, similarly too much or too little activity of these genes, and thus the reduced function of coded transcription factors may result in developing anterior segment defects and glaucoma. About 40% of ARM patients exhibited *FOXC1* gene mutations or duplications, *PITX2* gene mutations or deletions. To date 30 mutations of the *PITX2* gene have been described. The increased number of copies of *PITX2* gene may cause defects [1, 11].

The research has shown that the patients with *FOXC1* gene duplications usually presented IGD and frequently high intraocular pressure and glaucoma (appeared usually in childhood) than the patients with *FOXC1* gene mutations. Patients with *FOXC1* gene mutations presented more frequent disorders, such as iris hypoplasia, corectopia, peripheral synechiae of the anterior and posterior embryotoxon. Occurrence of high intraocular pressure and glaucoma was lower in the case of *FOXC1* gene mutation than *FOXC1* gene duplications, which suggests, that the patients with *FOXC1* gene duplications are more prone to glaucoma than those with *FOXC1* gene mutations. The presented results prove that the eye is especially susceptible to *FOXC1* gene duplications [11].

Recent research shows that *PAX6* gene controls the eyeball development in the whole animal kingdom, mainly through regulating of other genes expression during embryogenesis. *PAX6* gene abnormalities may cause aniridia or iris malformations similar to those in the patients with glaucoma. Multiple works have proven that the *PAX6* gene mutations which maps to chromosome 11p13 are connected to the heterogeneous group of anterior segment malformations including Peter's anomaly, autosomal dominant keratitis, congenital cataract, isolated foveal hypoplasia and aniridia [1]. The observations and assumptions concerning the role of *PAX6* gene were based on the tests conducted on mice. The mice that carried multiple wild copies of *PAX6* gene also exhibited eye malformations.

## CONCLUSIONS

The presented research results suggest, that genetic background play a crucial role in congenital glaucoma pathogenesis. Determining specific



genes or modifying factors would result in the wider knowledge of the disease, but it would also allow further development of diagnostic markers, screening tests and new treatment techniques.

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