## GENETIC BACKGROUND OF PRIMARY OPEN-ANGLE GLAUCOMA. PART I

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S u m m a r y. Glaucoma, a chronic degenerative optic neuropathy, is the second most common cause of blindness in the world. The appearance of the iridocorneal angle is crucial in the classification of the glaucoma, there are open-angle, close-angle and developmental categories, further which are divided into primary and secondary type. Scientists suggest that specific gene defects contribute to the pathogenesis of the glaucoma. Some of the glaucoma cases have Mendelian (dominant or recessive) inheritance pattern and is consistent with complex trait inheritance. This review gives an overview on the genetic aspects of glaucoma.

Key words: primary open-angle glaucoma, *GLC1A-N* genes, *MYOC* gene,

# GENETIC BACKGROUND OF PRIMARY OPEN-ANGLE GLAUCOMA

Primary open-angle glaucoma (POAG), previously known as "simple glaucoma", is presently the most common type of glaucoma. It is defined as the chronic, slowly progressing neuropathy of the optic nerve with the characteristic features of anatomical and functional damage. The progress of POAG is silent, painless and asymptomatic, resulting in irreversible damage to the optic nerve. The reduced visual field, being the result of progressing optic nerve damage, is initially unnoticeable to the patient. Frequently it is only the one eye blindness that brings the patient to the opthalmologist. The key factors leading to glaucoma include:

1) elevated intraocular pressure caused by the blockage of the aqueous humour outflow, mainly of the trabecular meshwork in the drainage angle, less frequently by the overproduction of the humour

2) decreased blood flow within the optic disc, resulting in ischaemia disrupting the ganglion cell metabolism; a cause of changes in the extracellular matrix within the lamina cribrosa which constitutes the scaffolding for the nerve fibres of the optic nerve.

However, at least a half of the open-angle glaucoma patients have intraocular pressure within the normal range, or even below the range. While diagnosing this form of glaucoma, the state of optic nerve disc should be considered, as well as the optic fibbers of the retina, only then followed by the level of the intraocular pressure and vision field.

POAG has been the best examined form of glaucoma as far as genetic liability is concerned. Numerous researchers have suggested its genetic background and conducted research that has allowed to identify several genes which were thought to contribute to its development. The greatest significance genes and the most deeply researched are discussed below.

## GLC1A-N CHROMOSOMAL LOCI

Numerous articles have shown the link between POAG and the described 14 loci defined as GLC1A-N; however, only 3 genes found there have been described in more detail: GLC1A – miocylina 7, GLC1E - optineuryna 9, and GLC1G -WDR36. Each of these genes is responsible for only a fraction of POAG cases, accounting for a small percentage of POAG, which may be inherited as a Mendelian trait, not as a set of traits. The genome research has contributed to identifying the genes of POAG susceptibility in adults [3].

The first POAG locus was identified among youths and adults. It was marked as GLC1A gene and is situated in the long arm of chromosome 1. The myocilin gene (*MYOC*) was definitively identified within the locus mentioned in 1997.

The second locus was described as GLC1B and identified within a region of 11.2 cM flanked by markers D2S2161 and D2S176 on chromosome 2cen-q13 [15]. The majority of patients with mutations within the locus show clinical symptoms, including low or medium IOP, the onset of the disease in later life (above the age of 40) and good response to treatment, which indicated that the GLC1B locus may encode the POAG gene, which is associated with normal or elevated IOP. Interestingly, in case of 8 families, whose members showed various clinical symptoms, the study did not reveal any link to the region [4].

GLC1C, the third locus discovered in POAG, is mapped to chromosome 3q21–q24 within a region of 11.1 cM between markers D3S3637 and D3S1744. It is linked to POAG, which is characterized by high levels of intraocular pressure (IOP), later onset of the disease (between the ages of 38 and 80) and moderate reaction to intraocular pressure-lowering medication [14]. Provided the defects in the extracellular matrix of the drainage angle may lead to glaucoma, the tests suggest a linkage between the abovementioned process and the *PCOLCE2* gene – type I procollagen C-proteinase enhancer protein as the gene presumably located within the GLC1C locus [16].

GLC1D, the fourth locus linked to adultonset POAG and high IOP levels, was discovered at the end of 1990. It was marked in the long arm of chromosome 8q23 within a region of 6.3 cM flanked by markers D8S1830 and D8S592 [15]. *SYDN2* is a gene presumably associated with the GLC1D locus, coding proteoglycans (proteins with large numbers of attached sugar chains) involved in cell surface interactions between molecules. It is assumed that abnormal forms of the protein could result in enhanced demyelination of the optic nerve fibres, increasing their sensitivity to an increase in intraocular pressure. As opposed to *SYND2*, the candidate gene, EGR-A, also associated with locus GLC1D, is a regulator gene, which by influencing the transcription (RNA production) of other glaucoma related genes, may disrupt their proper function [14].

The fifth locus was identified as GLC1E, within which the second POAG-related gene was discovered. This gene, *OPTN* (optic neuropathy-inducing protein) is mapped to chromosome 10p15-p14 within a region of 21 cM flanked between markers D10S1729 and D10S1664, and encodes the protein called optineurin. OPTN protein is found in the human trabecular meshwork, as well as in the retina, non-pigmented ciliary epithelium, and brain. This gene mutation are linked to adult-onset POAG and low-tension glaucoma. Since then, however, the approach to its perceived role in the pathogenesis of POAG has varied.

GLC1F is the sixth locus discovered in POAG, on the short arm of chromosome 7. It is associated with elevated IOP (22 mmHg to 38 mmHg). However, none of the genes localized within this area has been precisely defined. GLC1G is the seventh locus discovered on chromosome 5. The third adult-onset POAG gene, called WDR36, is found there. It is associated with high- and normaltension glaucoma. It encodes a protein present both in the eye tissues and in other tissues of the human organism. As with OPTN, its role in the POAG pathogenesis has not been clearly defined and fully examined. Two other POAG-related loci have been recently identified: GLC1H, on chromosome 2 associated with adult-onset glaucoma, and GLC1I, associated with adult early-onset glaucoma [2].

The genes examined so far, their presence, and the activity of their products may contribute to determining the POAG-inducing factors. These genes influence the clinical course of POAG, but in single cases, e.g. in the patients within the same family, who share the mutation, which, however, does not give certainty and is not always reflected by the severity of the disease. The development of POAG may thus vary in families with the same genetic mutations. The so-called modifier genes may influence the expression of some mutations of glaucoma. The role of modifier genes has not been examined and is still an enigma in many scientific circles [14]. The genes, that have been the most deeply explored and researched ones are described below.

### MYOC GENE

MYOC (TIGR, GLC1A) is a gene encoding the myocilin protein. It was first identified in POAG by Stone and his co-workers in 1997 and since then it has been examined and described by a wide group of scientists. Stone determined three mutations of the gene (Tyr430His, Gly357Val, Gln361Stop), detected in 4% of patients with familial glaucoma, 3% of glaucoma patients and sporadically in 0.3% of the general population [11]. The MYOC gene is located in GLC1A locus on chromosome 1 in the q21 region. It is composed of three exons and encodes 504 amino acids. Myocilin contains two hydrophobic regions, an elastic region connector and several potential areas of phosphorylation and glycosylation. The protein is primarily composed of an N-terminal region situated close to the coiled coil domain and the C-terminal olfactomedin domain. Olfactomedin is a component of a mucus layer surrounding the chemosensory dendrites of olfactory neurons in frogs. Glycoproteins related to a homology-bearing olfactomedin were found in the neurons of rats, mice and the human brain. The frog olfactomedin shares 31-40% of amino acid residue with MYOC, and human and rat olfactomedin-related glycoproteins share 46-50% of the amino acid residue with MYOC. However, if amino acid substitutions are taken into account. over 80% of the homologous olfactomedin-related glycoproteins can be found. The olfactomedin-like domain is primarily a disulphide beta bond between Cys245 and Cys433. It is suggested, that both the leucine zipper and the cysteine residue in amino acid 433 are involved in myocilin dimerisation and oligomerisation. The cysteine residues contributing to myocilin dimerisation are proteins homologous to olfactomedin, and its dimerisation and oligomerisation properties are likely to be crucial to the proper functioning of the MYOC protein [11]. The C-terminal region of MYOC contains three amino acids; serine, lysine and methionine, which have been proven to perform the peroxisomes functions.

Out of the three myocilin exons, the majority of mutations inducing the disease have been discovered mainly in the third exon, only a few in the first exon, while the second one has not been associated with any form of glaucoma. The third exon most frequently encodes the olfactomedin domains, in which the highest number of mutations is found, which contribute to the pathogenesis of POAG. The bioinformatic analysis has also proven that the olfactomedin domain present in the abnormal type or variant may be harmful to a large extent [11]. The myocilin mutations are dominant negative factors influencing the given mechanism of a particular function. A total of 66 point mutations are listed in the HGMD database (www.hgmd.cf.ac.uk/ac /index.php), but a fuller overview is available in the myocilin mutations database, where 74 nosological units induced by its mutation are described [11]. Around 40% of the mutations found are pathogenic, most of which (~85%) are missense mutations. It has been proven that approximately 1 in 30 POAG patients carries the disease-inducting mutation in MYOC gene, which accounts for between 2% and 5% of POAGrelated MYOC mutation cases worldwide. Myocilin mutations are typically linked with juvenile or early-onset adult POAG. These genetic forms of glaucoma are usually associated with elevated intraocular pressure and often require surgical intervention in order to stop the progression of the disease and alleviate the symptoms. In the adult POAG population the mutations of this gene occur in around 3-5% of patients with the late-onset form. A much greater percentage is evident among the young open angle glaucoma patients (JOAG, as well as the early-onset and severe forms of POAG) [9, 10]. The genetic connections of the gene described above make it responsible for a big proportion of hereditary glaucoma cases. The MYOC gene literature indicates that mutations in myocilin have a pathogenic influence mainly due to the inability to create correct proteins. When misfolded, i.e. mutated protein forms are created, they form aggregations in the endoplasmic reticulum (ER) (also called a Russell body), as well as in the cytoplasm (so-called aggresomes). Misfolded or unfolded proteins, unfolded protein response (UPR) in the cells, as well as the mitochondrial pathway of apoptosis initiated, which in result lead to cell death and the division of the trabecular meshwork (TM) cell structure, resulting in the blockage of the fluid passage, ocular hypertension and glaucoma [5,9]. Protein aggregations in the cell are a common symptom of many neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease and multiple sclerosis. It has been shown, that myocilin expression is present in many tissues vital for the development of glaucoma, e.g. on optic disc, astrocytes, aqueous humour (AQH), the meshwork trabeculae (TM), ciliary body and RCGs. For this reason it is thought, that the mutations in myocilin

may disturb the optic nerve function and the correct flow of the aqueous humour. However, no direct evidence has been found for that hypothesis [11].

The myocilin expression has been proven to be elevated also in scaring present in the glial cells, and its result is reflected by the inhibition of neurite growth. The myocilin mutations strongly contributed to mitochondrial membrane depolarization, decreased ATP production and reactive oxygen species production. It has been shown, that myocilin undergoes cleavage in the endoplasmic reticulum ER due to calpain II, an enzyme dependent on Ca level, into two fragments containing 20 kDa N-terminal domain and 35 kDa C-terminal domain of olfactomedin. The 35-kDa fragment and full myocilin cells were secreted into the extracellular matrix (ECM). The mutations in myocilin may disrupt this process and destruct the ECM structure within the trabecular meshwork (TM), which, in turn, leads to elevated IOP and glaucoma. It is hypothesized, that myocilin influences cell adhesion. It is described as Wnt signalling modulator, and it influences the adhesion properties of TM cells through pathways co-built by Rho GTPases and cAMP/ protein kinase [6]. Cell cultures with myocilin mutants have revealed and increased sensitivity to trypsin digestion. Thus, it may be concluded, that the myocilin mutants with altered adhesion properties interacting with other ECM proteins are responsible for the structural disintegration of TM cells.

The most frequent *MYOC* gene mutation is the Gln368Stop nonsense mutation, and this is the most frequent modification in the patients with adultonset POAG. For the patients with Gln368Stop mutation and diagnosed with glaucoma the average age is 59, and the maximum IOP – 30 mm Hg, while the Pro370Leu and Tyr437His mutations are associated with severe juvenile POAG. The patients with the Tyr437His mutations are diagnosed at the average age of 20, and the average maximum IOP for this group is 44 mmHg. Also, the treatment focused on lowering intraocular tension is often ineffective for Tyr437His, Val426Phe or 1177GACA>T patients and they typically require a procedure to improve filtration [1, 17].

Myocilin displays high expression in many tissues of the eyeball, such as the trabecular meshwork, sclera, iris, cornea, lens, ciliary body, retina, optic nerve and aqueous humour. Despite the proven significance of MYOC gene for glaucoma pathogenesis, its function (as a normal protein contained in the aqueous humour) has remained unknown. It has been postulated that MYOC protein facilitates the outflow of the aqueous humour through the trabecular meshwork, thus regulating the level of intraocular pressure [9, 12]. Recently, new theories have emerged as to the scheme of IOP increase in glaucoma induced by MYOC gene mutation. It has been suggested, that the products of the mutation require the Peroxisomal targeting Signal-1 receptor (PTS1R) to induce the intraocular pressure increase. It has been shown, that the human MYOC gene mutations may have caused the loss of the unknown sequences of targeting of peroxisomes, whose concurrence with PTS1R was necessary for the intraocular pressure increase [10]. However, the exact role of the correct MYOC protein and its link to the correct intraocular pressure, if it exists, remains unclear.

Other hypotheses suggest the stress defence role [9]. However, the initial allelic shortening or dropout from a correct gene is not significant for the disease pathogenesis. Null allele mice do not tend to develop high intraocular pressure leading to glaucoma. Also, following the histological analysis performed on these mice no irregularities were found of the structure responsible for the eye drainage, or even on the ultrastructural level [13]. The data indicates, that MYOC protein is not necessary for the normal intraocular pressure. The lack of glaucoma phenotypes in humans and null allele MYOC mice gene suggests, that the MYOC mutants are required for the disease to develop. Various groups have proven, that in vitro MYOC mutants create insoluble aggregations, which are not secreted, but gathered within the cells [8, 9]. The research has shown that the MYOC mutants may induce ER stress and cell death when the TM cells are grown in the normal body temperature [8]. However, if the cells are grown in a lower temperature, which facilitates protein folding and their secretion, the cells have increased resistance. This suggests, that misfolded pathogenic proteins are accumulated. TM cells seem more sensitive than other cell types, which is possibly caused by the fact that a specific type of processing of the mutants is conducted within these cells [8]. Higher susceptibility of the TM cells increases the probability of glaucoma. Such a toxic mechanism also suggests, that the normal functioning of the remaining glaucoma genes may have little to do

with the physiological homeostasis of intraocular pressure. These findings suggest, that the processes facilitating protein folding and secretion may have a positive impact on the intraocular pressure. Since ER stress frequently induces apoptosis, antiapoptotic processes may also be beneficial thanks to decreased cell death.

The wild-type myocilin overexpression is also believed to be involved in glaucoma pathogenesis. However, contradictory opinions on the subject still exist. It is believed, that in the case of steroid-induced glaucoma, myocilin overexpression is one of the triggering factors for glaucoma causation. It is widely recognised, that myocilin contains several GRE in the promoter region, which is why its overexpression in TM cells related to dexamethasone, glucocorticosteroid treatment was observed. Myocilin is also known as TIGR (trabecular meshwork inducible glucocorticosteroid response) [7].

## CONCLUSIONS

The current research suggests, that myocilin may influence the integrity of the TM structure, protein oversecretion or acquired defects caused by mutations. The structural defects of TM, possible damage to the optic nerve resulting from unknown processes lead to glaucoma.

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