

Review

Biomarkers in primary open angle glaucoma

Haris Kokotas^{1,2,*}, Christos Kroupis³, Dimitrios Chiras⁴, Maria Grigoriadou¹, Klea Lamnissou², Michael B. Petersen¹ and George Kitsos⁴

¹Department of Genetics, Institute of Child Health, 'Aghia Sophia' Children's Hospital, Athens, Greece

²Department of Genetics and Biotechnology, Faculty of Biology, University of Athens, Athens, Greece

³Department of Clinical Biochemistry, Attikon Hospital, Athens University Medical School, Athens, Greece

⁴Department of Ophthalmology, Ioannina University Medical School, Ioannina, Greece

Abstract

Glaucoma, a leading cause of blindness worldwide, is currently defined as a disturbance of the structural or functional integrity of the optic nerve that causes characteristic atrophic changes in the optic nerve, which may lead to specific visual field defects over time. This disturbance usually can be arrested or diminished by adequate lowering of intraocular pressure (IOP). Glaucoma can be divided roughly into two main categories, 'open angle' and 'closed angle' glaucoma. Open angle, chronic glaucoma tends to progress at a slower rate and patients may not notice loss of vision until the disease has progressed significantly. Primary open angle glaucoma (POAG) is described distinctly as a multifactorial optic neuropathy that is chronic and progressive with a characteristic acquired loss of optic nerve fibers. Such loss develops in the presence of open anterior chamber angles, characteristic visual field abnormalities, and IOP that is too high for the healthy eye. It manifests by cupping and atrophy of the optic disc, in the absence of other known causes of glaucomatous disease. Several biological markers have been implicated with the disease. The purpose of this study was to summarize the current knowledge regarding the non-genetic molecular markers which have been predicted to have an association with POAG but have not yet been validated.

Keywords: aqueous humor; biomarker; factor; intraocular pressure; primary open angle glaucoma; trabecular meshwork.

Introduction

The incidence of glaucoma is one in 200 people aged 50 or younger, and one in 10 people aged 80 or older. It is an eye disorder in which the optic nerve suffers damage which affects vision in the eye(s) and if untreated leads to permanent impairment of the optic nerve and resultant visual field loss, which can progress to blindness. The nerve damage involves loss of retinal ganglion cells in a characteristic pattern. It is often, but not always, associated with increased pressure of the fluid in the anterior cavity of the eye (aqueous humor, AH) (1). With pharmaceutical and/or surgical intervention it is possible to halt further loss of vision. Glaucoma has been nicknamed the 'silent thief of sight' because the loss of vision normally occurs gradually over a long period of time and is often only recognized when the disease is quite advanced. Worldwide, it is the second leading cause of blindness, only second to cataract. It is also the leading cause of blindness among Africans (2).

The definition of glaucoma has changed drastically over the years. There are many different sub-types of glaucoma but they can all be generally considered as optic neuropathy. Increased intraocular pressure (IOP) (above 21 mm Hg or 2.8 kPa) is a risk factor associated with the development of the disease, but is not the disease itself. Nevertheless, some controversy still exists as to whether IOP should be included in the definition, as some subsets of patients can exhibit the characteristic optic nerve damage and visual field defects while having an IOP within the normal range. One person may develop nerve damage at a relatively low pressure, while another person may have high eye pressure for years and still not develop damage. Patients can develop optic neuropathy of glaucoma in the absence of documented elevated IOP. This condition has been termed normal- or low-tension glaucoma. People who maintain elevated pressures in the absence of nerve damage or visual field loss also exist. They are considered at risk for glaucoma and have been termed glaucoma suspects or ocular hypertensives.

In many patients with glaucoma, IOP elevation is associated with an increase in resistance to fluid leaving the eye (Figure 1), an abnormality thought to occur primarily at the level of fluid passage through the trabecular meshwork (TM) and Schlemm's canal (3, 4). In a minority of cases the increase in outflow resistance is caused by restricted access of AH to the TM (angle closure or narrow-angle glaucoma) or to a mechanical clogging of the meshwork (e.g., pigmentary glaucoma). However, in primary open angle glaucoma (POAG), the angle appears normal and the cause for the increase in outflow resistance through the outflow pathway, especially

*Corresponding author: Haris Kokotas, PhD, Department of Genetics, Institute of Child Health, 'Aghia Sophia' Children's Hospital, Athens 11527, Greece
Phone: +30 213 2037333, Fax: +30 210 7700111,
E-mail: hkokotas@yahoo.gr

Received January 22, 2012; accepted May 20, 2012;
previously published online June 29, 2012

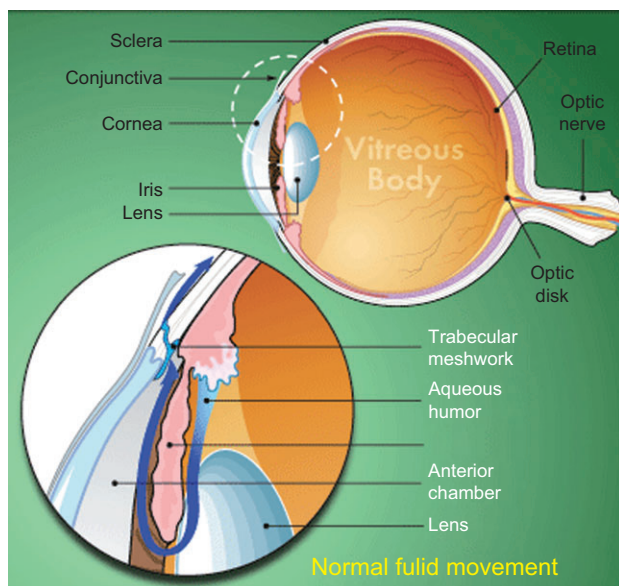


Figure 1 Normal fluid movement in the eye.

Aqueous humor flows from the posterior to anterior chambers through the pupil and is drained through the trabecular meshwork in a healthy eye. The cause for the increase in resistance through the outflow pathway in POAG has remained obscure (Figure taken from <http://static.howstuffworks.com/gif/glaucoma-diagram.gif>).

the cell- and connective tissue-filled lattice work of the TM, has remained obscure (5).

It is predicted that by the year 2020 POAG will affect more than 50 million people worldwide (6). It is the most common form of glaucoma, with reported prevalence rates ranging from 1.1% (7) to 3.8% (8). POAG is a long-term ocular disease process that is generally bilateral and often asymmetric. It is defined by optic disc or retinal nerve fiber structural abnormalities and visual field abnormality as detected by optic disc examination and visual field testing. POAG is suggested to be a neurodegenerative disorder which is triggered by different factors including mechanical stress due to intraocular pressure, reduced blood flow to retina, reperfusion injury, oxidative stress, glutamate excitotoxicity, and aberrant immune response. The most definitive evidence of glaucoma is documented, as progressive change in optic disc appearance and reproducible worsening in automated visual field testing. Structural alterations of the optic nerve or nerve fiber layer occur more frequently prior to visual field abnormalities or visual defects, although the opposite may be also seen. There is strong evidence that IOP plays an important role in the neuropathy of POAG, and it has been shown that a reduction in the level of IOP lessens the risk of visual field progression in open-angle glaucoma. Apart from IOP, other risk factors include old age, race, female gender, myopia, corneal thickness, optic disc hemorrhages, hemodynamics, and positive family history (9). Since POAG is a chronic condition, it must be monitored for life, but early diagnosis is the first and crucial step to preserve vision. Many factors have been found to be implicated with the disease, including genetic and non-genetic markers. At the genetic level, a distinction between

juvenile onset (age at diagnosis 10–35 years) and adult onset (age at diagnosis above 35 years) has been made. Many juvenile onset cases have autosomal dominant inheritance whilst adult onset cases are typically multifactorial. POAG is caused by multiple genetic and environmental factors, as well as their interactions. Mutations, polymorphisms, and copy number variations (CNVs) could contribute to the pathogenesis of POAG. To date, more than 20 genetic loci have been implicated in its development. Linkage analysis has identified two POAG-causing genes, myocilin (*MYOC*) and optineurin (*OPTN*). More than 70 *MYOC* mutations have been reported to contribute to the pathogenesis of POAG, and *OPTN* mutations have been associated with normal tension glaucoma. Variants in these two genes account for about 5% of POAG in the population. Previous studies also have reported the association of POAG with mutations in WD repeat domain 36 (*WDR36*) and neurotrophin-4 (*NTF4*); however, their roles in the pathogenesis of POAG is controversial. CNVs are defined as insertions or deletions of large segments of DNA, from 1 kb up to several Mb; they have been found to contribute to many complex disorders (10).

Biomarkers are characteristics that are specifically measured with adequate accuracy and precision and evaluated as indicators of normal biological or pathogenic processes, or to monitor pharmacologic responses to a therapeutic intervention. Thus, biomarkers might be invaluable tools to identify individuals at risk for disease and, depending on the approach, could serve to measure the outcomes of therapies. Biomarkers are molecules with biologically important intra- or intercellular function, an expression or activity of which either causes or is specifically altered in response to corresponding pathologic condition (11) and they are measured in the laboratory (e.g., protein concentration in urine). Molecules can change qualitatively (e.g., in case of gene mutations) or quantitatively (e.g., in case of an altered gene expression). The role of biomarkers in medicine is to specify molecular alterations/reactions/pathways attributable to concrete pathologic condition. Generally, biomarkers should not be necessarily linked to any genetic mutation; both mRNA expression and protein levels possess valuable information about cellular response to distinct pathologic conditions, and, therefore, are frequently used for specific biomarker selection. Indeed, the most important criterion for a biomarker is its disease specificity. Whereas alteration in expression status of only one gene is almost never disease specific, a gene expression profile could be particularly valuable for the creation of highly precise diagnostic approaches (11). The development of clinically useful biomarkers is an area of active investigation and includes genetic screening tests, proteomic markers, and analyses of serum antibodies to retina and optic nerve proteins. The purpose of the present study is to summarize all the current information regarding these factors that have so far been suggested to be related with the diagnosis of the disease but have not been established and represent biomarker candidates to be validated. These markers, measured in blood (serum or plasma), AH, or tissues, alone or in combination with each other, could be crucial for the early diagnosis of POAG, or even for the prevention of the disease in individuals who are at higher risk. Genetic markers

of POAG, including gene expression and mutations/polymorphisms have been excluded from this study, as they have been broadly reviewed recently (12–14). Antibodies and proteomic markers have been listed in alphabetical order.

Biomarkers in POAG

3 α -HSD

3 α -Hydroxysteroid dehydrogenases (3 α -HSDs) catalyze the conversion of 3-ketosteroids to 3 α -hydroxy compounds. The best known 3 α -HSD activity is the transformation of the most potent natural androgen, dihydrotestosterone, into 5 α -androstan-3 α ,17 β -diol (3 α -diol), a compound having much lower activity. 3 α -HSDs could play a crucial role in the control of a series of active steroid levels in target tissues. In the human, type 1 3 α -HSD was first identified as human chlordecone reductase (15). 3 α -HSDs are members of the aldo-keto reductase family and catalyze the conversion of 3-ketosteroids to the corresponding 3 α -hydroxy compounds using NADPH as the cofactor.

Weinstein et al. determined whether peripheral blood lymphocytes from POAG patients have reduced 3 α -HSD activity (16). The results of this study showed an association of decreased peripheral blood lymphocytes 3 α -HSD activity and POAG which was not related to antiglaucoma therapy. The authors concluded that the reduced levels of 3 α -HSD activity in the readily obtainable peripheral blood lymphocytes may serve as a marker for POAG or those at risk for developing the disease.

ANGPTL7

ANGPTL7 is a member of the angiopoietin-like (ANGPTL) family of proteins that have high sequence and structural homology to the angiopoietins, which are important regulators of angiogenesis (17). All ANGPTL proteins studied to date have been shown to be involved in blood vessel formation or neovascularization in several models, including corneal angiogenesis assays (18, 19). In addition, ANGPTL proteins have been demonstrated to play an important role in lipid metabolism by inhibition of phospholipase (20). ANGPTL proteins appear to be bi-functional, with both functions demonstrated for most of the family members studied. ANGPTL7 has been identified by expression microarray analysis to be a highly induced mRNA in response to either dexamethasone or transforming growth factor- β (TGF- β) treatment of TM cells in vitro (21, 22).

ANGPTL7 was also identified as possibly being associated with POAG in a proteomics study of TM tissue (23). Kuchtey et al. found that the concentration of ANGPTL7 was elevated in the AH of POAG eyes, supporting the notion that ANGPTL7 could be involved in POAG pathogenesis (24). They concluded that elevated TGF- β_2 levels in glaucomatous AH could cause increased ANGPTL7 expression, which in turn could induce collagen changes, or through other mechanisms, contribute to the pathogenesis of POAG.

Antibodies

By enzyme-linked immunosorbent assay (ELISA), Tezel and coworkers (25) compared the serum immunoreactivity to glycosaminoglycans, and by immunohistochemistry they compared the distribution patterns of glycosaminoglycans in the optic nerve head of POAG eyes vs. controls. The authors found that these autoantibodies may increase the susceptibility of the optic nerve head to damage in these patients by changing the functional properties of the lamina cribrosa, its vasculature, or both (25).

The glutathione S-transferase (GST) supergene family, which encodes detoxification enzymes, is widely expressed in mammalian tissue cytosols and membranes. GST is present in glial and neuronal cells of the central nervous system and in the retina (26). Increased titers of autoantibodies to GST in some patients with POAG may represent a generalized response to tissue stress and/or damage as a consequence of the glaucomatous neurodegeneration process and thereby secondary production of serum antibodies to GST in the glaucomatous retina (26).

Approximately 20% of POAG patients possess a serum antibody against neuron specific enolase (NSE), and the maximum IOP levels in POAG patients with anti-NSE antibody are statistically lower than those without the antibody (27). It has been suggested that the anti-NSE antibody can reach the retina through circulation and cause retinal ganglion cell damage and progression of visual field loss in addition to elevated IOP (28), and that the presence of serum autoantibody against NSE may be clinically useful for predicting the progression of visual field loss in POAG patients (29).

Several studies have presented data regarding the association of a number of antibodies related to POAG and other glaucomatous groups. Among them, the serum titers of antibodies against heat shock proteins (30, 31), and the entire IgG autoantibody patterns against different retina, optic nerve, and optic nerve head antigens in sera have been investigated (32–37). Another study (38) was carried out to investigate the levels of anti-*Helicobacter pylori* IgG antibodies in the AH and serum of POAG patients. A significant increase of *H. pylori* IgG antibody levels was demonstrated, suggesting that the titer of anti-*H. pylori* IgG antibody in the AH might reflect the severity of glaucomatous damage in POAG patients. Yuki et al. analyzed the serum of POAG patients for *Chlamydia pneumoniae* and *Chlamydia trachomatis* immunoglobulin G antibody titers by ELISA, and found significantly higher immunoglobulin G titers for *C. pneumoniae* in POAG patients than in controls (39). This may indicate either a common factor that causes susceptibilities to both glaucoma and *C. pneumoniae* infection, or that *C. pneumoniae* may be a causal factor for developing POAG (39).

AP₄A

Diadenosine tetraphosphate (AP₄A) is a compound that contains two adenosine moieties bridged by four phosphates. AP₄A is found in tears, heart, and brain (40). Oxidative stress induces synthesis of AP₄A (41). In vivo, AP₄A reduced

translocation of mitochondrial cytochrome C, activation of cytoplasmic caspase-3, and cerebral infarction in ischemic cerebral cortex in vivo (42). These data suggest that AP₄A is protective, via anti-apoptotic mechanisms, against ischemic injury in cerebral cortex. AP₄A also has positive effects on dopaminergic neurons. Selective AP₄A binding sites were found in substantia nigra and striatum (43, 44). The role of AP₄A in POAG has been investigated in one recent study (40). AP₄A was detected in human AH and its concentrations were significantly elevated in POAG patients compared to controls.

BDNF

Brain-derived neurotrophic factor (BDNF) is one of the polypeptide growth factors known to be vital components for building up and preserving of neurons. BDNF is transported to the retinal ganglion cell bodies through a retrograde axonal transportation system and the synaptic connections within (45). BDNF in the tears of normal tension glaucoma patients is significantly less than normal, and this may be a potential diagnostic biomarker for early detection of normal tension glaucoma. BDNF crosses the blood-brain barrier and as a result, the level of this factor in the blood can relatively reflect its concentration in the brain. BDNF levels in serum were determined in POAG patients and controls by ELISA (45). The authors concluded that BDNF in the serum might be a useful biochemical marker for early detection of POAG.

Caspase-14

Caspase-14 is a unique member of the evolutionarily conserved family of cysteinyl aspartate-specific proteinases, which are mainly involved in inflammation and apoptosis. Although most caspases are ubiquitously expressed, caspase-14 expression is confined mainly to cornifying epithelia, such as the skin. Moreover, caspase-14 activation correlates with cornification, indicating that it plays a role in terminal keratinocyte differentiation. According to a recent study, caspase-14 might be involved in the apoptosis of ocular tissues in POAG by either directly mediating or inducing caspase-8 and caspase-9 activation in vitro (46).

CD44H

CD44 is an 80 to 90 kDa, type 1, transmembrane multifunctional glycoprotein, and it is the principal receptor of the glycosaminoglycan, hyaluronan (47, 48). CD44 is expressed in a wide variety of cell types, including mature T-cells, B-cells, medullary thymocytes, granulocytes, macrophages and fibroblasts, and the corneal epithelium (49) and retina (50, 51). CD44 binds to the actin cytoskeleton, mediates cell attachment to the extracellular matrix (52), and participates in fibroblast migration in provisional wound healing (53) and in immunologic activation (54). CD44 participates in the uptake and degradation of hyaluronan (55). POAG is associated with a decreased content of hyaluronan in the TM and in the juxtacanalicular connective tissue. Knepper and co-workers (56)

examined selected regions of the anterior segment to localize and determine the content of CD44H. Their results indicate that CD44H may represent a marker of POAG and an etiologic factor in the POAG disease process. The ectodomain of CD44 is shed as a 32 kDa fragment-soluble CD44 (sCD44) which is cytotoxic to TM cells and retinal ganglion cells in culture (57). It has been shown that sCD44 adversely affects retinal ganglion cells and TM cell survival in vitro, by activating a proapoptotic pathway (58). More recent studies (59–61) have also demonstrated that sCD44 in AH could be a potential biomarker for POAG.

Cellular senescence

Cellular senescence has been hypothesized to constitute an antagonistic pleiotropic response that protects against cancer early in life, but has cumulative deleterious effects, contributing to aging and certain age-related diseases (62). Acquisition of a senescent phenotype can result from either multiple rounds of cell proliferation in vitro (replicative senescence) (63), or by exposure to different types of stress factors (stress-induced premature senescence) (64). Since the proliferation rate of TM cells is very low (65), it is more likely that acquisition of a senescent phenotype in the glaucomatous outflow pathway may result from stress-induced premature senescence rather than from the exhaustion of their replicative potential. One factor that could potentially contribute to stress-induced premature senescence in the TM is the constant exposure of TM cells to an oxidative environment (66, 67). An additional factor that could contribute to the observed increased presence of senescent cells in the outflow pathway from POAG donors is the reported increased resistance to apoptosis associated with the acquisition of a senescence phenotype (68), which may favor the survival of senescent cells and lead to their accumulation in the outflow pathway over time. The increased production of reactive oxygen species by senescent cells could potentially lead to an increase in apoptosis of the adjacent non-senescent cells, and therefore contribute to the decrease in the absolute number of cells observed in glaucoma (69). POAG is associated with a significant increase in the number of senescent cells in the outflow pathway. Given the multiple potential adverse effects that these senescent cells might have on outflow pathway function, it has been hypothesized that cellular senescence might serve as a biomarker and could contribute to the increase in AH outflow resistance and IOP commonly associated with POAG (70).

Cystatin C

Cystatins, and in particular cystatin C, have been shown to be involved in many biological events and have not always been related to protease inhibition; examples include a neural stem cell factor, osteoclast differentiation, pathophysiological process in brain ischemia as well as in atherosclerotic plaque development (71). In a study by Duan and co-workers, a significant increase of cystatin C was observed in the AH of POAG patients (46). The increase was similar to the changes in cerebrospinal fluid of Alzheimer's disease,

suggesting that POAG shares similar mechanisms with Alzheimer's disease.

Cytokines

Cytokines are secreted proteins that play central roles in modulating immunity, but they can also perform non-immune functions in areas such as angiogenesis and development. If immune activation is associated with glaucoma, changes in cytokine secretion within the eye might be detectable as changes in the concentration of cytokines in the AH of glaucoma patients (72). Cytokines include interleukins, interferons, colony-stimulating factors, chemokines, tumor necrosis factor, and growth factors. Tumor necrosis factor α (TNF- α), a macrophage/monocyte derived pluripotent cytokine, is associated with tissue ischemia, neuronal damage, and remodeling (73), and increased levels signify neuronal damage after brain trauma. In humans, the TNF- α expression is elevated in the optic nerve and the retina of glaucomatous eyes (73–75). Significant alterations of serum cytokines are associated with glaucoma (72, 76, 77), suggesting the possibility that abnormal immune environments contribute to the glaucomatous neuropathy of POAG.

Transforming growth factors (TGFs) constitute a family of multifunctional polypeptides of approximately 25 kDa, and exhibit pleiotropic regulatory actions upon most vertebral cell types (78, 79). Depending on the cell type, they regulate proliferation, migration, differentiation, cytokine production, synthesis of extracellular matrix, wound healing, immunosuppression, and in vivo angiogenesis (80). Transforming growth factor- β (TGF- β) exists in at least five genetically distinct isoforms, β_1 – β_5 (81). Among these, only three isoforms, namely β_1 , β_2 , and β_3 are expressed in human ocular tissues (81). TGF- β_2 is regarded as the major isoform in the eye (78, 80, 81). Elevated levels of TGF- β_2 have been detected in the AH of glaucomatous eyes (81–84). A distinct structural change in the TM of patients with POAG is the increase in fibrillar extracellular matrix in the juxtacanalicular region of the TM. TGF- β_2 signaling may be involved, as TGF- β_2 is significantly increased in the AH of patients with POAG. In cultured human TM cells, TGF- β_2 causes an increase in extracellular matrix deposition (85). The concentration of TGF- β_2 has been previously measured in several studies (86–88). The results have shown that the levels of total TGF- β_2 in the aqueous samples of POAG are elevated and have suggested that minimizing TGF- β_2 levels may help to prevent the aging process in the TM as seen in POAG (88). Extracellular matrix elasticity modulates TGF- β -induced signaling and protein expression in human TM cells. Increasing extracellular matrix elasticity in vitro promotes protein expression patterns reminiscent of patterns reported in POAG. Therefore, changes in TM elasticity and mechanical load may have a significant role in the disease (89).

Erythropoietin

Human erythropoietin (EPO) is an acidic glycoprotein hormone with a molecular mass of 34 kDa. As the prime

regulator of red cell production, its major functions are to promote erythroid differentiation and to initiate hemoglobin synthesis. Sakanaka et al. reported in vivo evidence that EPO protects neurons against ischemia-induced cell death (90). They presented findings suggesting that EPO may exert its neuroprotective effect by reducing the nitric oxide-mediated formation of free radicals or antagonizing their toxicity. Siren and colleagues presented data suggesting that inhibition of neuronal apoptosis underlies short latency protective effects of EPO after cerebral ischemia and other brain injuries (91). In rats, Junk et al. conducted parallel studies of recombinant EPO in a model of transient global retinal ischemia induced by raising intraocular pressure, which is a clinically relevant model for retinal diseases. They observed abundant expression of the EPO receptor (EPOR) throughout the ischemic retina (92). Becerra and Amaral (93) hypothesized that identification and separation of the structural determinants within the erythropoietin molecule could elucidate additional ways to minimize side effects associated with local administration of erythropoietin to the eye, an approach that offers advantages over systemic administration. Recently, the role of AH erythropoietin in POAG has been investigated, and the studies have concluded that erythropoietin is a possible biomarker for the disease (61, 94, 95).

GRP78

The 78 kDa glucose-regulated protein (GRP78) is an abundant multi-functional protein that binds to endocytosomal reticulum stress transducers and serves as a transmitter in altering of endocytosomal reticulum homeostasis. These proteins originally generate a cytoprotective signal leading to reduced translation, then improve endocytosomal reticulum protein folding capacity, and clear misfolded endocytosomal reticulum proteins (96). Studies performed during the last decade identified GRP78 as a ubiquitous luminal resident protein of the endocytosomal reticulum that plays a key role in assisting the corrected folding of protein tertiary and quaternary structures. Recent studies indicated a close connection between GRP78 and endocytosomal reticulum stress in certain disease processes (97). GRP78 was down-regulated in TM cells of POAG patients compared to TM cells of healthy controls, even when treated with an endocytosomal reticulum stress inducer (97).

Hepcidin

The Hepcidin prohormone (Hep) is a small peptide produced in the liver. Human Hep is produced from an 84 amino acid precursor, including a putative single peptide. Hep is an important peptide hormone that plays a critical role in the regulation of iron efflux from numerous cell types, including intestinal cells, macrophages, and hepatocytes (98). A recent study found that Hep is expressed in Müller cells, photoreceptor cells, and retinal pigmented epithelium. The expression of Hep in the retina points to the local intraocular regulation of iron metabolism, separate from a dependence on the circulation liver-derived hormone: circulating Hep would likely

be inaccessible to intraocular tissues due to the presence of blood-ocular barriers (99). Recently, it was shown that local Hep secretions may have a pathogenic role in POAG (100), however, the authors raised a number of questions that need to be elucidated before final conclusions are drawn.

Homocysteine

Homocysteine (Hcy) is an amino acid of interest which has cytotoxic and vasculopathic actions such as apoptosis of retinal ganglion cells, extracellular matrix alterations, oxidative stress, and ischemic vascular dysregulation (101). Available evidence indicates that Hcy is directly toxic to neurons and blood vessels and can induce DNA strand breakage, oxidative stress, and apoptosis. The methioninehomocysteine metabolic pathway intermediaries, S-adenosylmethionine and S-adenosylhomocysteine, produce methyl groups required for the synthesis of catecholamines and DNA methylation. Since Hcy is a sensitive indicator of vitamin B deficiency, an elevated Hcy level has been suggested as a marker for a pathogenic process (102). Recent reports on Hcy measured in AH, plasma, and tear fluid have yielded mixed results with some studies demonstrating an association with normal tension glaucoma, pseudoexfoliative glaucoma, and POAG, whilst others have not (101, 103). Hcy metabolism is complex with both genetic and environmental factors. Differences in these factors could, in part, account for the disparity in reported results. Vitamin B12, vitamin B6, and folate act as cofactors in Hcy metabolism (101).

Hydroxyproline

Hydroxyproline (Hyp) is an imino acid normally present in human plasma. It is derived primarily from endogenous collagen turnover and the breakdown of dietary collagen. The finding of elevated (5- to 10-fold increase from the normal of <50 μ mol) serum hydroxyproline is thought to be an inherited defect in the catabolism of hydroxyproline. Hydroxyproline is produced by hydroxylation of the amino acid proline by the enzyme prolyl hydroxylase following protein synthesis (as a post-translational modification). The enzyme catalyzed reaction takes place in the lumen of the endoplasmic reticulum. Although it is not directly incorporated into proteins, hydroxyproline comprises roughly 4% of all amino acids found in animal tissue, more than seven amino acids which are directly incorporated (104). Recently, the levels of Hyp were assessed by spectrometry in the AH and plasma of human eyes with POAG (103). This study demonstrated that Hyp levels were significantly higher in the AH of POAG patients compared to controls, while no significant difference between the levels of Hyp in plasma of POAG and controls was shown (103).

Myocilin

Myocilin, a 55–57 kDa secreted glycoprotein and member of the olfactomedin family is expressed in many ocular and non-ocular tissues. The protein is found in both intracellular and extracellular spaces, and has been linked to elevations

in intraocular pressure. TM issue from POAG, low-tension glaucoma, and pseudoexfoliation glaucoma had increased myocilin immunoreactivity when compared to normal tissue (105). Steroid treatment of monolayer TM cells or cultured human anterior segments showed a time and pressure dependent increase in myocilin protein production and secretion. Perfusion of human anterior segment organ culture with recombinant myocilin increases outflow resistance (105). Furthermore, animal models show a correlation between increased myocilin levels and elevated IOP. Rats that spontaneously develop increased IOP had a 4-fold increase in myocilin transcription. Myocilin is present in AH where it may serve as a marker for the glaucomatous state (105). Evidence supports a role for elevated myocilin levels in canine glaucomatous AH particularly in breeds with primary glaucoma. Less well understood is the relationship of myocilin levels in human glaucomatous AH, particularly in POAG and pseudoexfoliation glaucoma. In humans, a trend towards elevated myocilin levels in human glaucomatous AH has been reported, however, limited sample size prevented a glaucoma sub-type classification (106). Misfolded mutant myocilin forms secretion-incompetent intracellular aggregates. The block of myocilin secretion was proposed to alter the extracellular matrix environment of the TM, with subsequent impediment of AH outflow leading to elevated intraocular pressure. Endoplasmic reticulum stress-induced apoptosis could be a pathway to explain the reduction of TM cells in patients with myocilin-caused glaucoma. As a consequence, the phagocytotic capacity of the remaining TM cell population would be insufficient for effective cleaning of AH, constituting a major pathogenetic factor for the development of increased intraocular pressure in POAG (107). Myocilin levels were tested in control AH and compared to the levels of POAG and pseudoexfoliation glaucoma AH to determine whether levels of myocilin are altered in these glaucoma subsets. These levels were significantly elevated in human POAG AH when compared with control AH (105). A more recent study demonstrated that myocilin expression is not altered in the blood of POAG patients, unlike myocilin expression in the corresponding TM cultures (108). These results suggested that myocilin expression is not altered systemically but rather that myocilin expression may contribute to POAG pathogenesis in specific tissues such as TM.

Nitric oxide synthase

In blood vessels, a key regulator of resistance changes is the intercellular (and sometimes intracellular) gaseous modulator, nitric oxide (NO) (109). Production of vascular NO is regulated in several ways: by circulating hormones acting on endothelial cell NO synthase (eNOS); by non-adrenergic, non-cholinergic, perivascular nerves acting through neuronal NOS (bNOS; NOS-1); by blood flow acting through mechanical distortion of vessels (shear stress); and by cytokines and perhaps other factors acting on inducible NOS (iNOS) present in vascular smooth muscle. Increased synthesis of NO leads to smooth muscle relaxation, increase in luminal diameter, and a reduction in vascular resistance. Although studies (110)

have reported that rats demonstrate little NOS-1 activity in their ocular outflow pathway, an enrichment (relative to other ocular tissues) of eNOS (NOS-3) in the human outflow system (TM, Schlemm's canal, and collecting channels) and in the ciliary muscle, especially its anterior longitudinal portion has been shown (111). The long ciliary muscle is known to send tendinous insertions into the TM and, by contraction and relaxation, to affect the complex TM anatomy and to alter outflow resistance (112). Direct topical or intracameral application of NO agonists has been reported to alter outflow facility (113, 114), and some clinical reports have indicated that systemic administration of the NO-mimicking nitrovasodilators can lower IOP at doses that do not alter systemic blood pressure. Interestingly, the magnitude of the IOP decrease observed in such cases has sometimes been observed to be greater in patients with POAG than in patients with ocular normal-tension or in those with angle-closure glaucoma. In another study, the authors (115) hypothesized that *in vivo* iNOS overexpression in the chronic progress of POAG could contribute to TM cell damage, through protein nitration by reactive peroxynitrite. This process can be an important link in the chain of events leading to the oxidative damage observed in severe POAG (116, 117). Furthermore, the increased nitrotyrosine in severe POAG may serve as a marker of oxidative stress in the progression of cell death in POAG (115).

PGDS

Prostaglandin H2 D-isomerase (PGDS) is responsible for the biosynthesis of prostaglandin D2 in the central nervous system. It is a bifunctional protein that acts as both a retinoid transporter and a prostaglandin D2-producing enzyme (118). Elevated PGDS levels have been observed in the serum of patients with renal impairment, diabetes mellitus, and hypertension. Recently, the ability of PGDS to induce apoptosis in a variety of cell types including epithelial cells, neuronal cells, and vascular smooth muscle cells was demonstrated (119). Therefore, it was suggested that PGDS might mediate the apoptosis of TM. A significant increase of PGDS in the AH of POAG patients compared to controls has been proved by Western blotting analysis (46).

Phospholipase A₂

Phospholipase A₂ (PLA₂) belongs to a superfamily of enzymes that catalyzes the hydrolysis of the sn-2 ester bond in phospholipids. The hydrolysis products are free fatty acids and lysophospholipids. Different PLA₂ isoenzymes have been found and classified into several groups (from I to XIV) based on their structures, subcellular distributions, cellular functions, and enzymatic characteristics (120). The PLA₂ concentration of tears was measured with time-resolved fluoroimmunoassay in patients with senile cataract and patients with POAG, and when compared with the PLA₂ concentration of tears in healthy controls, no significant differences were found (121). However, recently it was shown that secretory PLA₂-IIA (sPLA₂-IIA) is overexpressed in the TM of POAG eyes. This result supports the hypothesis that oxidative stress may play a

significant role in the pathogenesis of POAG. sPLA₂-IIA has been proposed as an inflammatory marker of cardiovascular disease, and therefore, higher expression of macrophage-derived sPLA₂-IIA in POAG compared to normal controls supports the view that vascular diseases and POAG may share common pathophysiological mechanisms (122).

Transferrin

Transferrin is a member of a large family of iron binding proteins that transports ferric iron and possibly zinc between the sites of absorption, storage, and utilization. Because transferrin is present in blood at a concentration that is approximately 200-fold higher than in AH, it is a particularly good marker for studying the integrity of the blood-aqueous barrier. In combination with other growth modulating substances, transferrin regulates the growth and maintenance of many cells of the anterior segment of the eye *in vivo* and *in vitro*, and it also has been implicated in the pathophysiologic changes of glaucoma (123). However, despite the presence of transferrin and its receptor-binding sites in intraocular tissues, the lack of a significant proliferative activity in the tissues that border the anterior chamber of the eye probably can be attributed to the bioavailability of transferrin to its target cells. This process might depend on circulating transferrin receptors shed by the cells that maintain the capacity to bind transferrin and on the interactive effects of other growth-modulating substances that, not only regulate the expression of transferrin receptors on the cell surface, but also are increased quantitatively in AH (123). Elevated transferrin has been observed in the AH of POAG patients, which has been attributed to elevated IOP (46).

Transthyretin

Transthyretin (TTR) is a tetrameric plasma protein and usually responsible for the transportation of thyroxine and retinol (124). Sometimes TTR and its variants lead to extracellular polymerization of insoluble protein fibrils called amyloid deposits. The mechanism of the accumulation is still not known. Findings of increased levels of TTR in the AH of glaucoma patients indicate that this protein might play a role in the pathogenesis of glaucoma. Interfering protein precipitations of TTR might cause a mechanical barrier for aqueous fluid that could consequently lead to glaucoma in some patients. There are some theoretical explanations about elevated TTR levels in glaucomatous AH. In animal experiments, TTR synthesis in ciliary pigment epithelium was shown (125). This location of TTR synthesis could explain the local influence of this protein in the anterior chamber of the eye without having a systemic effect. Another study on rats showed a widespread ocular distribution of TTR including retinal pigment epithelium and retinal ganglion cells (126). Possibly dying retinal ganglion cells in glaucoma patients dispense their TTR in the AH, which leads to higher TTR concentrations in patients. Transthyretin has been identified in studies of rheumatoid arthritis, Alzheimer's disease, and POAG (127), and significant differences in the concentration of TTR in the AH between POAG patients and controls were found (46, 127).

Table 1 Classification and comparison of the candidate biomarkers discussed in this review.

Candidate biomarker	Type	Measured in
3 α -HSD	Enzyme	Blood
ANGPTL7	Protein	TM, AH
Antibody for glycosaminoglycans	Antibody	Serum
Antibody for GST	Antibody	Serum
Antibody for NSE	Antibody	Serum
Antibody for heat shock proteins	Antibody	Serum
Antibody for anti- <i>Helicobacter pylori</i>	Antibody	AH, serum
Antibody for <i>Chlamydia pneumoniae</i>	Antibody	Serum
AP ₄ A	Compound nucleotide	AH
BDNF	Protein	Serum
Caspase-14	Enzyme	AH
CD44H	Protein	TM, AH
Cellular senescence	Antagonistic pleiotropic response	AH
Cystatin C	Protein	AH
Cytokines	Proteins	AH, TM, serum
Erythropoietin	Hormone	AH
GRP78	Protein	TM
Hepcidin	Hormone	AH
Homocysteine	Amino acid	AH, plasma, tear fluid
Hydroxyproline	Imino acid	AH, plasma
Myocilin	Protein	AH, TM
Nitric oxide synthase	Enzyme	TM, Schlemm's canal and collecting channels
PGDS	Enzyme	AH, TM, serum
Phospholipase A ₂	Enzyme	TM
Transferrin	Protein	AH
Transthyretin	Protein	AH

Conclusions – expert opinion

In this review we summarized the cumulative knowledge regarding the biomarkers which have been associated with POAG but remain to be validated in the preferred detection tissue for the disease. Molecular markers including antibodies and proteomic markers have been discussed, while genetic markers have been excluded due to the fact that they have been extensively reviewed recently. These markers are compared and classified in Table 1. Specific sensitive screening tools, including these biomarkers, are needed to effectively identify individuals who will benefit from therapy. It will be important to carefully select patients who will respond better to targeted therapies (e.g., cell based and viral gene transfer therapies). It is also important that treatment should be initiated when it will have the best impact on the disease. Technologies that will make it possible to monitor damaged ganglion cells at stages when they can still be rescued need to be developed. In vivo imaging of retinal cell apoptosis suggests that in the future it could be possible to identify ganglion cells entering apoptosis in POAG patients. Such advancement will also make it possible to determine the efficacy of therapy. We can expect that a patient's risk for POAG will be established using a combination of genetic, clinical and biochemical markers, an assessment of ganglion cell disease will be made by novel imaging techniques, and appropriate therapy will be initiated to restore ganglion cell health. The use of the biomarkers discussed here and of those which will be identified in the future,

will allow early disease detection and timely therapy targeted to molecular disease mechanisms, significantly improving the quality of life of POAG patients.

Highlights

- Primary open-angle glaucoma is described distinctly as a multifactorial optic neuropathy that is chronic and progressive with a characteristic acquired loss of optic nerve fibers.
- A biomarker is specifically measured with adequate accuracy and precision and evaluated as an indicator of normal biological or pathogenic processes, or to monitor pharmacologic responses to a therapeutic intervention.
- Biomarkers can be useful tools to identify individuals at risk and to measure the outcomes of therapies.
- Biomarkers measured in blood, aqueous humor, or tissues, alone or in combination with each other, could be crucial for the early diagnosis of primary open-angle glaucoma.
- The analytical value of the molecular tests for the detection of the discussed biomarkers will be guaranteed by validation of the relevant methods.

Conflict of interest statement

Authors' conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article.

Research funding: None declared.

Employment or leadership: None declared.

Honorarium: None declared.

References

- Thomassen TL, Perkins ES, Dobree JH. Aqueous veins in glaucomatous eyes. *Br J Ophthalmol* 1950;34:221–7.
- WHO, Worldwide Health Organization, <http://www.who.int/bulletin/volumes/82/11/feature1104/en/index.html>. Accessed on 14 May, 2012.
- Grierson I, Calthorpe CM. Characteristics of meshwork cells and age changes in the outflow system of the eye: their relevance to primary open-angle glaucoma. In: Mills KB, editor. *Glaucoma*. New York: Pergamon, 1988:12–31.
- Rohen JW, Lutjen-Drecoll E. Morphology of aqueous outflow pathways in normal and glaucomatous eyes. In: Ritch R, Shields M, Krupin T, editors. *The glaucomas*. Philadelphia: CV Mosby, 1989:41–74.
- Nathanson JA, McKee M. Alterations of ocular nitric oxide synthase in human glaucoma. *Invest Ophthalmol Vis Sci* 1995;36:1774–84.
- Quigley HA, Broman AT. The number of people with glaucoma worldwide in 2010 and 2020. *Br J Ophthalmol* 2006;90:262–7.
- Dielemans I, Vingerling JR, Wolfs RC, Hofman A, Grobbee DE, de Jong PT. The prevalence of primary open-angle glaucoma in a population-based study in the Netherlands. *The Rotterdam Study. Ophthalmology* 1994;101:1851–5.
- Topouzis F, Wilson MR, Harris A, Anastasopoulos E, Yu F, Mavroudis L, et al. Prevalence of open-angle glaucoma in Greece: the Thessaloniki Eye Study. *Am J Ophthalmol* 2007;144:511–9.
- Golubnitschaja O, Yeghiazaryan K, Flammer J. Key molecular pathways affected by glaucoma pathology: is predictive diagnosis possible? *EPMA* 2010;1:237–44.
- Dong S, Yang J, Yu W, Kota P, Xia X, Xu H. No association of genetic polymorphisms in CYP1B1 with primary open-angle glaucoma: a meta- and gene-based analysis. *Mol Vis* 2012;18:786–96.
- Golubnitschaja O, Flammer J. What are the biomarkers for glaucoma? *Surv Ophthalmol* 2007;52(Suppl 2):S155–61.
- Ray K, Mookherjee S. Molecular complexity of primary open angle glaucoma: current concepts. *J Genet* 2009;88:451–67.
- Fan BJ, Wiggs JL. Glaucoma: genes, phenotypes, and new directions for therapy. *J Clin Invest* 2010;120:3064–72.
- Fingert JH. Primary open-angle glaucoma genes. *Eye* 2011;25:587–95.
- Dufort I, Labrie F, Luu-The V. Human types 1 and 3 α -hydroxysteroid dehydrogenases: differential lability and tissue distribution. *J Clin Endocrinol Metab* 2001;86:841–6.
- Weinstein BI, Iyer RB, Binstock JM, Hamby CV, Schwartz IS, Moy FH, et al. Decreased 3 α -hydroxysteroid dehydrogenase activity in peripheral blood lymphocytes from patients with primary open angle glaucoma. *Exp Eye Res* 1996;62:39–45.
- Katoh Y, Katoh M. Comparative integromics on Angiopoietin family members. *Int J Mol Med* 2006;17:1145–9.
- Camenisch G, Pisabarro MT, Sherman D, Kowalski J, Nagel M, Hass P, et al. ANGPTL3 stimulates endothelial cell adhesion and migration via integrin α v β 3 and induces blood vessel formation in vivo. *J Biol Chem* 2002;277:17281–90.
- Oike Y, Ito Y, Maekawa H, Morisada T, Kubota Y, Akao M, et al. Angiopoietin-related growth factor (AGF) promotes angiogenesis. *Blood* 2004;103:3760–5.
- Sukonina V, Lookene A, Olivecrona T, Olivecrona G. Angiopoietin-like protein 4 converts lipoprotein lipase to inactive monomers and modulates lipase activity in adipose tissue. *Proc Natl Acad Sci USA* 2006;103:17450–5.
- Lo WR, Rowlette LL, Caballero M, Yang P, Hernandez MR, Borrás T. Tissue differential microarray analysis of dexamethasone induction reveals potential mechanisms of steroid glaucoma. *Invest Ophthalmol Vis Sci* 2003;44:473–85.
- Zhao X, Ramsey KE, Stephan DA, Russell P. Gene and protein expression changes in human trabecular meshwork cells treated with transforming growth factor-beta. *Invest Ophthalmol Vis Sci* 2004;45:4023–34.
- Bhattacharya SK, Rockwood EJ, Smith SD, Bonilha VL, Crabb JS, Kuchtey RW, et al. Proteomics reveal Cochlin deposits associated with glaucomatous trabecular meshwork. *J Biol Chem* 2005;280:6080–4.
- Kuchtey J, Källberg ME, Gelatt KN, Rinkoski T, Komáromy AM, Kuchtey RW. Angiopoietin-like 7 secretion is induced by glaucoma stimuli and its concentration is elevated in glaucomatous aqueous humor. *Invest Ophthalmol Vis Sci* 2008;49:3438–48.
- Tezel G, Edward DP, Wax MB. Serum autoantibodies to optic nerve head glycosaminoglycans in patients with glaucoma. *Arch Ophthalmol* 1999;117:917–24.
- Yang J, Tezel G, Patil RV, Romano C, Wax MB. Serum autoantibody against glutathione S-transferase in patients with glaucoma. *Invest Ophthalmol Vis Sci* 2001;42:1273–6.
- Maruyama I, Ohguro H, Ikeda Y. Retinal ganglion cells recognized by serum autoantibody against gamma-enolase found in glaucoma patients. *Invest Ophthalmol Vis Sci* 2000;41:1657–65.
- Maruyama I, Ikeda Y, Nakazawa M, Ohguro H. Clinical roles of serum autoantibody against neuron-specific enolase in glaucoma patients. *Tohoku J Exp Med* 2002;197:125–32.
- Ikeda Y, Maruyama I, Nakazawa M, Ohguro H. Clinical significance of serum antibody against neuron-specific enolase in glaucoma patients. *Jpn J Ophthalmol* 2002;46:13–7.
- Tezel G, Seigel GM, Wax MB. Autoantibodies to small heat shock proteins in glaucoma. *Invest Ophthalmol Vis Sci* 1998;39:2277–87.
- Wax MB, Tezel G, Kawase K, Kitazawa Y. Serum autoantibodies to heat shock proteins in glaucoma patients from Japan and the United States. *Ophthalmology* 2001;108:296–302.
- Grus FH, Joachim SC, Hoffmann EM, Pfeiffer N. Complex autoantibody repertoires in patients with glaucoma. *Mol Vis* 2004;10:132–7.
- Joachim SC, Pfeiffer N, Grus FH. Autoantibodies in patients with glaucoma: a comparison of IgG serum antibodies against retinal, optic nerve, and optic nerve head antigens. *Graefes Arch Clin Exp Ophthalmol* 2005;243:817–23.
- Grus FH, Joachim SC, Bruns K, Lackner KJ, Pfeiffer N, Wax MB. Serum autoantibodies to alpha-fodrin are present in glaucoma patients from Germany and the United States. *Invest Ophthalmol Vis Sci* 2006;47:968–76.
- Joachim SC, Wuenschig D, Pfeiffer N, Grus FH. IgG antibody patterns in aqueous humor of patients with primary open angle glaucoma and pseudoexfoliation glaucoma. *Mol Vis* 2007;13:1573–9.
- Reichelt J, Joachim SC, Pfeiffer N, Grus FH. Analysis of autoantibodies against human retinal antigens in sera of patients with glaucoma and ocular hypertension. *Curr Eye Res* 2008;33:253–61.
- Joachim SC, Reichelt J, Berneiser S, Pfeiffer N, Grus FH. Sera of glaucoma patients show autoantibodies against myelin basic protein and complex autoantibody profiles against human optic nerve antigens. *Graefes Arch Clin Exp Ophthalmol* 2008;246:573–80.

38. Kountouras J, Mylopoulos N, Konstas AG, Zavos C, Chatzopoulos D, Boukla A. Increased levels of *Helicobacter pylori* IgG antibodies in aqueous humor of patients with primary open-angle and exfoliation glaucoma. *Graefes Arch Clin Exp Ophthalmol* 2003;241:884–90.
39. Yuki K, Kimura I, Shiba D, Imamura Y, Tsubota K. Elevated serum immunoglobulin G titers against *Chlamydia pneumoniae* in primary open-angle glaucoma patients without systemic disease. *J Glaucoma* 2010;19:535–9.
40. Castany M, Jordi I, Catala J, Gual A, Morales M, Gasull X, et al. Glaucoma patients present increased levels of diadenosine tetraphosphate, Ap(4)A, in the aqueous humour. *Exp Eye Res* 2011;92:221–6.
41. Bochner BR, Lee PC, Wilson SW, Cutler CW, Ames BN. AppppA and related adenylated nucleotides are synthesized as a consequence of oxidation stress. *Cell* 1984;37:225–32.
42. Wang Y, Chang CF, Morales M, Chiang YH, Harvey BK, Su TP, et al. Diadenosine tetraphosphate protects against injuries induced by ischemia and 6-hydroxydopamine in rat brain. *J Neurosci* 2003;23:7958–65.
43. Pintor J, Miras-Portugal MT. A novel receptor for diadenosine polyphosphates coupled to calcium increase in rat midbrain synaptosomes. *Br J Pharmacol* 1995;115:895–902.
44. Oaknin S, Rodríguez-Ferrer CR, Aguilar JS, Ramos A, Rotllán P. Receptor binding properties of di (1,N6-ethenoadenosine) 5', 5''-P1, P4-tetraphosphate and its modulatory effect on extracellular glutamate levels in rat striatum. *Neurosci Lett* 2001;309:177–80.
45. Ghaffariyeh A, Honarpisheh N, Heidari MH, Puyan S, Abasov F. Brain-derived neurotrophic factor as a biomarker in primary open-angle glaucoma. *Optom Vis Sci* 2011;88:80–5.
46. Duan X, Xue P, Wang N, Dong Z, Lu Q, Yang F. Proteomic analysis of aqueous humor from patients with primary open angle glaucoma. *Mol Vis* 2010;16:2839–46.
47. Entwistle J, Hall CL, Turley EA. HA receptors: regulators of signalling to the cytoskeleton. *J Cell Biochem* 1996;61:569–77.
48. Sherman L, Sleeman J, Dall P, Hekele A, Moll J, Ponta H, et al. The CD44 proteins in embryonic development and in cancer. *Curr Top Microbiol Immunol* 1996;213(Pt 1):249–69.
49. Kaya G, Rodriguez I, Jorcano JL, Vassalli P, Stamenkovic I. Selective suppression of CD44 in keratinocytes of mice bearing an antisense CD44 transgene driven by a tissue-specific promoter disrupts hyaluronate metabolism in the skin and impairs keratinocyte proliferation. *Genes Dev* 1997;11:996–1007.
50. Chaitin MH, Wortham HS, Brun-Zinkernagel AM. Immunocytochemical localization of CD44 in the mouse retina. *Exp Eye Res* 1994;58:359–65.
51. Nishina S, Hirakata A, Hida T, Sawa H, Azuma N. CD44 expression in the developing human retina. *Graefes Arch Clin Exp Ophthalmol* 1997;235:92–6.
52. Cohen MB, Griebing TL, Ahaghotu CA, Rokhlin OW, Ross JS. Cellular adhesion molecules in urologic malignancies. *Am J Clin Pathol* 1997;107:56–63.
53. Svec K, White J, Vaillant P, Jessurun J, Roongta U, Krumwiede M, et al. Acute lung injury fibroblast migration and invasion of a fibrin matrix is mediated by CD44. *J Clin Invest* 1996;98:1713–27.
54. Naor D, Sionov RV, Ish-Shalom D. CD44: structure, function, and association with the malignant process. *Adv Cancer Res* 1997;71:241–319.
55. Culty M, Nguyen HA, Underhill CB. The hyaluronan receptor (CD44) participates in the uptake and degradation of hyaluronan. *J Cell Biol* 1992;116:1055–62.
56. Knepper PA, Goossens W, Mayanil CS. CD44H localization in primary open-angle glaucoma. *Invest Ophthalmol Vis Sci* 1998;39:673–80.
57. Knepper PA, Miller AM, Choi J, Wertz RD, Nolan MJ, Goossens W, et al. Hypophosphorylation of aqueous humor sCD44 and primary open-angle glaucoma. *Invest Ophthalmol Vis Sci* 2005;46:2829–37.
58. Choi J, Miller AM, Nolan MJ, Yue BY, Thotz ST, Clark AF, et al. Soluble CD44 is cytotoxic to trabecular meshwork and retinal ganglion cells in vitro. *Invest Ophthalmol Vis Sci* 2005;46:214–22.
59. Nolan MJ, Giovengo MC, Miller AM, Wertz RD, Ritch R, Liebmann JM, et al. Aqueous humor sCD44 concentration and visual field loss in primary open-angle glaucoma. *J Glaucoma* 2007;16:419–29.
60. Budak YU, Akdogan M, Huysal K. Aqueous humor level of sCD44 in patients with degenerative myopia and primary open-angle glaucoma. *BMC Res Notes* 2009;2:224.
61. Mokbel TH, Ghanem AA, Kishk H, Arafa LF, El-Baiomy AA. Erythropoietin and soluble CD44 levels in patients with primary open-angle glaucoma. *Clin Experiment Ophthalmol* 2010;38:560–5.
62. Campisi J. Senescent cells, tumor suppression, and organismal aging: good citizens, bad neighbors. *Cell* 2005;120:513–22.
63. Hayflick L, Moorhead PS. The serial cultivation of human diploid cell strains. *Exp Cell Res* 1961;25:585–621.
64. Toussaint O, Royer V, Salmon M, Remacle J. Stress-induced premature senescence and tissue ageing. *Biochem Pharmacol* 2002;64:1007–9.
65. Rohen JW, Lutjen-Drecoll E. Biology of the trabecular meshwork. In: Lutjen-Drecoll E, editor. *Basic aspects of glaucoma research*. Schattauer Verlag; Stuttgart, 1982:141–66.
66. Garcia-Castineiras S, Velazquez S, Martinez P, Torres N. Aqueous humor hydrogen peroxide analysis with dichlorophenol – indophenol. *Exp Eye Res* 1992;55:9–19.
67. Spector A, Ma W, Wang RR. The aqueous humor is capable of generating and degrading H₂O₂. *Invest Ophthalmol Vis Sci* 1998;39:1188–97.
68. Wang E. Senescent human fibroblasts resist programmed cell death, and failure to suppress bcl2 is involved. *Cancer Res* 1995;55:2284–92.
69. Alvarado J, Murphy C, Juster R. Trabecular meshwork cellularity in primary open-angle glaucoma and nonglaucomatous normals. *Ophthalmology* 1984;91:564–79.
70. Liton PB, Challa P, Stinnett S, Luna C, Epstein DL, Gonzalez P. Cellular senescence in the glaucomatous outflow pathway. *Exp Gerontol* 2005;40:745–8.
71. Bäcklund A, Holmdahl M, Mattsson R, Håkansson K, Lindström V, Nandakumar KS, et al. Cystatin C influences the autoimmune but not inflammatory response to cartilage type II collagen leading to chronic arthritis development. *Arthritis Res Ther* 2011;13:R54.
72. Kuchty J, Rezaei KA, Jaru-Ampornpan P, Sternberg P Jr, Kuchty RW. Multiplex cytokine analysis reveals elevated concentration of interleukin-8 in glaucomatous aqueous humor. *Invest Ophthalmol Vis Sci* 2010;51:6441–7.
73. Balaiya S, Edwards J, Tillis T, Khetpal V, Chalam KV. Tumor necrosis factor-alpha (TNF-a) levels in aqueous humor of primary open angle glaucoma. *Clin Ophthalmol* 2011;5:553–6.
74. Yuan L, Neufeld AH. Tumor necrosis factor-alpha: a potentially neurodestructive cytokine produced by glia in the human glaucomatous optic nerve head. *Glia* 2000;32:42–50.

75. Tezel G, Li LY, Patil RV, Wax MB. TNF-alpha and TNF-alpha receptor-1 in the retina of normal and glaucomatous eyes. *Invest Ophthalmol Vis Sci* 2001;42:1787-94.
76. Sawada H, Fukuchi T, Tanaka T, Abe H. Tumor necrosis factor-alpha concentrations in the aqueous humor of patients with glaucoma. *Invest Ophthalmol Vis Sci* 2010;51:903-6.
77. Huang P, Qi Y, Xu YS, Liu J, Liao D, Zhang SS, et al. Serum cytokine alteration is associated with optic neuropathy in human primary open angle glaucoma. *J Glaucoma* 2010;19:324-30.
78. Kokawa N, Sotozono C, Nishida K, Kinoshita S. High total TGF-beta 2 levels in normal human tears. *Curr Eye Res* 1996;15:341-3.
79. Imanishi J, Kamiyama K, Iguchi I, Kita M, Sotozono C, Kinoshita S. Growth factors: importance in wound healing and maintenance of transparency of the cornea. *Prog Retin Eye Res* 2000;19:113-29.
80. Roberts AB, Sporn MB. The transforming growth factor- β s. In: Sporn MB, Roberts AB, editors. *Handbook of experimental pharmacology*. Heidelberg: Springer-Verlag, 1990;95:419-72.
81. Nishida K, Sotozono C, Adachi W, Yamamoto S, Yokoi N, Kinoshita S. Transforming growth factor-beta 1, -beta 2 and -beta 3 mRNA expression in human cornea. *Curr Eye Res* 1995;14:235-41.
82. Jampel HD, Roche N, Stark WJ, Roberts AB. Transforming growth factor- β in human aqueous humor. *Eye Res* 1990;9:963-9.
83. Tanihara H, Inatani M, Honda Y. Growth factor and their receptors in the retina and pigment epithelium. *Prog Ret Eye Res* 1997;16:271-301.
84. Inatani M, Tanihara H, Katsuta H, Honjo M, Kido N, Honda Y. Transforming growth factor-beta 2 levels in aqueous humor of glaucomatous eyes. *Graefes Arch Clin Exp Ophthalmol* 2001;239:109-13.
85. Fuchshofer R, Stephan DA, Russell P, Tamm ER. Gene expression profiling of TGFbeta2- and/or BMP7-treated trabecular meshwork cells: identification of Smad7 as a critical inhibitor of TGF-beta2 signaling. *Exp Eye Res* 2009;88:1020-32.
86. Ochiai Y, Ochiai H. Higher concentration of transforming growth factor-beta in aqueous humor of glaucomatous eyes and diabetic eyes. *Jpn J Ophthalmol* 2002;46:249-53.
87. Min SH, Lee TI, Chung YS, Kim HK. Transforming growth factor-beta levels in human aqueous humor of glaucomatous, diabetic and uveitic eyes. *Korean J Ophthalmol* 2006;20:162-5.
88. Yu AL, Birke K, Moriniere J, Welge-Lüssen U. TGF- β 2 induces senescence-associated changes in human trabecular meshwork cells. *Invest Ophthalmol Vis Sci* 2010;51:5718-23.
89. Han H, Wecker T, Grehn F, Schlunck G. Elasticity-dependent modulation of TGF- β responses in human trabecular meshwork cells. *Invest Ophthalmol Vis Sci* 2011;52:2889-96.
90. Sakanaka M, Wen TC, Matsuda S, Masuda S, Morishita E, Nagao M, et al. In vivo evidence that erythropoietin protects neurons from ischemic damage. *Proc Natl Acad Sci USA* 1998;95:4635-40.
91. Sirén AL, Fratelli M, Brines M, Goemans C, Casagrande S, Lewczuk P, et al. Erythropoietin prevents neuronal apoptosis after cerebral ischemia and metabolic stress. *Proc Natl Acad Sci USA* 2001;98:4044-9.
92. Junk AK, Mammis A, Savitz SI, Singh M, Roth S, Malhotra S, et al. Erythropoietin administration protects retinal neurons from acute ischemia-reperfusion injury. *Proc Natl Acad Sci USA* 2002;99:10659-64.
93. Becerra SP, Amaral J. Erythropoietin – an endogenous retinal survival factor. *N Engl J Med* 2002;347:1968-70.
94. Cumurcu T, Bulut Y, Demir HD, Yenisehirli G. Aqueous humor erythropoietin levels in patients with primary open-angle glaucoma. *J Glaucoma* 2007;16:645-8.
95. Wang ZY, Zhao KK, Zhao PQ. Erythropoietin is increased in aqueous humor of glaucomatous eyes. *Curr Eye Res* 2010;35:680-4.
96. Shen X, Zhang K, Kaufman RJ. The unfolded protein response – a stress signaling pathway of the endoplasmic reticulum. *J Chem Neuroanat* 2004;28:79-92.
97. Chai F, Luo R, Li Y, Bai Y, He Y, Wei Y, et al. Down-regulation of GRP78 in human glaucomatous trabecular meshwork cells. *Mol Vis* 2010;16:1122-31.
98. Nemeth E, Tuttle MS, Powelson J, Vaughn MB, Donovan A, Ward DM, et al. Heparin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. *Science* 2004;306:2090-3.
99. Gnana-Prakasam JP, Martin PM, Mysona BA, Roon P, Smith SB, Ganapathy V. Heparin expression in mouse retina and its regulation via lipopolysaccharide/Toll-like receptor-4 pathway independent of Hfe. *Biochem J* 2008;411:79-88.
100. Sorkhabi R, Ghorbanihaghjo A, Javadzadeh A, Motlagh BF, Ahari SS. Aqueous humor heparin pro-hormone levels in patients with primary open angle glaucoma. *Mol Vis* 2010;16:1832-6.
101. Turgut B, Kaya M, Arslan S, Demir T, Guler M, Kaya MK. Levels of circulating homocysteine, vitamin B6, vitamin B12, and folate in different types of open-angle glaucoma. *Clin Interv Aging* 2010;5:133-9.
102. Ebesunun MO, Eruvulobi HU, Olagunju T, Owuoye OA. Elevated plasma homocysteine in association with decreased vitamin B(12), folate, serotonin, lipids and lipoproteins in depressed patients. *Afr J Psychiatry (Johannesburg)* 2012;15:25-9.
103. Ghanem AA, Mady SM, El Awady HE, Arafa LF. Homocysteine and hydroxyproline levels in patients with primary open-angle glaucoma. *Curr Eye Res* 2012 Mar 29 [Epub ahead of print].
104. Gorres KL, Raines RT. Prolyl 4-hydroxylase. *Crit Rev Biochem Mol Biol* 2010;45:106-24.
105. Howell KG, Vrabel AM, Chowdhury UR, Stamer WD, Fautsch MP. Myocilin levels in primary open-angle glaucoma and pseudoexfoliation glaucoma human aqueous humor. *J Glaucoma* 2010;19:569-75.
106. Jacobson N, Andrews M, Shepard AR, Nishimura D, Searby C, Fingert JH, et al. Non-secretion of mutant proteins of the glaucoma gene myocilin in cultured trabecular meshwork cells and in aqueous humor. *Hum Mol Genet* 2001;10:117-25.
107. Yam GH, Gaplovska-Kysela K, Zuber C, Roth J. Aggregated myocilin induces russell bodies and causes apoptosis: implications for the pathogenesis of myocilin-caused primary open-angle glaucoma. *Am J Pathol* 2007;170:100-9.
108. Abu-Amro KK, Azad TA, Spaeth GL, Myers J, Katz LJ, Moster M, et al. Unaltered myocilin expression in the blood of primary open angle glaucoma patients. *Mol Vis* 2012;18:1004-9.
109. Moncada S, Palmer RM, Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol Rev* 1991;43:109-42.
110. Yamamoto R, Bredt DS, Snyder SH, Stone RA. The localization of nitric oxide synthase in the rat eye and related cranial ganglia. *Neuroscience* 1993;54:189-200.
111. Nathanson JA, McKee M. Identification of an extensive system of nitric oxide-producing cells in the ciliary muscle and outflow pathway of the human eye. *Invest Ophthalmol Vis Sci* 1995;36:1765-73.

112. Flügel C, Bárány EH, Lütjen-Drecoll E. Histochemical differences within the ciliary muscle and its function in accommodation. *Exp Eye Res* 1990;50:219–26.
113. Nathanson JA. Nitric oxide and nitrovasodilators in the eye: implications for ocular physiology and glaucoma. *J Glaucoma* 1993;2:206–10.
114. Schuman JS, Erickson K, Nathanson JA. Nitrovasodilator effects on intraocular pressure and outflow facility in monkeys. *Exp Eye Res* 1994;58:99–105.
115. Fernández-Durango R, Fernández-Martínez A, García-Feijoo J, Castillo A, de la Casa JM, García-Bueno B, et al. Expression of nitrotyrosine and oxidative consequences in the trabecular meshwork of patients with primary open-angle glaucoma. *Invest Ophthalmol Vis Sci* 2008;49:2506–11.
116. Izzotti A, Sacca SC, Cartiglia C, De Flora S. Oxidative deoxyribonucleic acid damage in the eyes of glaucoma patients. *Am J Med* 2003;114:638–46.
117. Saccà SC, Pascotto A, Camicione P, Capris P, Izzotti A. Oxidative DNA damage in the human trabecular meshwork: clinical correlation in patients with primary open-angle glaucoma. *Arch Ophthalmol* 2005;123:458–63.
118. Tanaka T, Urade Y, Kimura H, Eguchi N, Nishikawa A, Hayaishi O. Lipocalin-type prostaglandin D synthase (beta-trace) is a newly recognized type of retinoid transporter. *J Biol Chem* 1997;272:15789–95.
119. Ragolia L, Palaia T, Paric E, Maesaka JK. Elevated L-PGDS activity contributes to PMA-induced apoptosis concomitant with downregulation of PI3-K. *Am J Physiol Cell Physiol* 2003;284:C119–26.
120. Six DA, Dennis EA. The expanding superfamily of phospholipase A(2) enzymes: classification and characterization. *Biochim Biophys Acta* 2000;1488:1–19.
121. Aho VV, Nevalainen TJ, Paavilainen V, Saari KM. Group II phospholipase A2 content of tears in patients with senile cataract and primary open-angle glaucoma. *Eur J Ophthalmol* 2002;12:40–3.
122. Rönkkö S, Rekonen P, Kaarniranta K, Puustjarvi T, Teräsvirta M, Uusitalo H. Phospholipase A2 in chamber angle of normal eyes and patients with primary open angle glaucoma and exfoliation glaucoma. *Mol Vis* 2007;13:408–17.
123. Tripathi RC, Borisuth NS, Tripathi BJ, Gotsis SS. Quantitative and qualitative analyses of transferrin in aqueous humor from patients with primary and secondary glaucomas. *Invest Ophthalmol Vis Sci* 1992;33:2866–73.
124. Monaco HL, Rizzi M, Coda A. Structure of a complex of two plasma proteins: transthyretin and retinol-binding protein. *Science* 1995;268:1039–41.
125. Kawaji T, Ando Y, Nakamura M, Yamamoto K, Ando E, Takano A, et al. Transthyretin synthesis in rabbit ciliary pigment epithelium. *Exp Eye Res* 2005;81:306–12.
126. Dwork AJ, Cavallaro T, Martone RL, Goodman DS, Schon EA, Herbert J. Distribution of transthyretin in the rat eye. *Invest Ophthalmol Vis Sci* 1990;31:489–96.
127. Grus FH, Joachim SC, Sandmann S, Thiel U, Bruns K, Lackner KJ, et al. Transthyretin and complex protein pattern in aqueous humor of patients with primary open-angle glaucoma. *Mol Vis* 2008;14:1437–45.



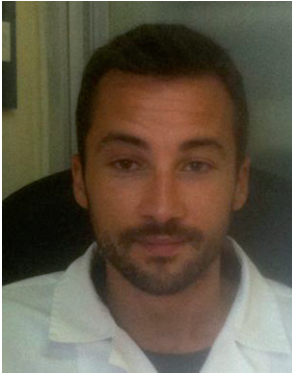
Dr. Haris Kokotas received his BSc from the Faculty of Biology, University of Athens, Greece and earned his PhD in Human Molecular Genetics from the Athens University Medical School. He has gained experience on cytogenetic and molecular techniques and he has focused his research on the genetics of the head, including eye diseases and hearing loss. He is currently a postdoctoral fellow at the Department of Genetics, Institute of Child Health, Athens, Greece and is the principal investigator or co-investigator in several research projects. He has presented in several conferences worldwide and has published numerous articles in national and international peer-reviewed scientific journals.

He is currently a postdoctoral fellow at the Department of Genetics, Institute of Child Health, Athens, Greece and is the principal investigator or co-investigator in several research projects. He has presented in several conferences worldwide and has published numerous articles in national and international peer-reviewed scientific journals.



Dr. Christos Kroupis obtained a MSc from Cornell University in Ithaca, NY, USA for his work on *T. fusca* mutants and a PhD from University of Athens, Greece for molecular studies in *BRCA* genes. Before becoming a Lecturer of Clinical Biochemistry and Molecular Diagnostics in the University of Athens in 2006 and then an Asst. Professor in 2011, he has worked as a researcher for Hoffmann-La

Roche, NJ, USA (basic cardiovascular research) and as a staff Clinical Chemist and Molecular Biologist for Onassis Cardiac Center and Mitera Surgical Center for over 12 years. His research concerns biomarkers for cervical neoplasia, *FCGR2A* polymorphisms in HIT and cardiac disease, genetics of glaucoma and macular degeneration and *PALB2* genetics in breast cancer patients. He serves also as a Lead Accessor in the National Accreditation System (E.S.Y.D.) and as a National Representative in EFCC.



Dimitrios Chiras was born in 1982 in Ioannina (Epirus, Greece). In 1999 he entered the School of Medicine and Surgery, University of Bologna, Italy and graduated with honors (100/110) in 2005. Following examinations, in 2006 he acquired the right to practice medicine in Italy and subsequently did 3 months of practical training in Internal Medicine, Surgery and General Medicine at Sant'Orsola Hospital in Bologna. In 2007 he enrolled at the Ophthalmological Clinic of the University Hospital of Ioannina, and since 2008 he is preparing a doctoral dissertation on the "Evaluation of APOE, MTHFR and LOXL1 polymorphisms in pseudoexfoliation syndrome and pseudoexfoliation glaucoma in Epirus (Greece)." In 2009 he began his residency in ophthalmology at the Agios Savvas Hospital in Athens. He has participated in many conferences concerning ophthalmological points of interest.



Maria Grigoriadou obtained her BSc degree in Biology from the University of Thessaloniki, Greece. Since 1989 she has worked at the Department of Genetics, Institute of Child Health, Athens, Greece, initially as a cytogeneticist (1989–1996) and later as a molecular biologist (1996-to date). Her main scientific interests include chromosomal abnormalities, genetics of hearing loss and genetics of blindness.



Dr. Klea Lamnissou obtained a BSc-Biology and a PhD-Genetics from the University of Athens, Greece (Faculty of Biology). She is Associate Professor of Human Molecular Genetics in the Department of Genetics and Biotechnology, Faculty of Biology, University of Athens. Her research concerns identification of genes, gene polymorphisms or mutations that are implicated in

various human diseases. Research in the laboratory is mainly focusing on association studies in polygenic and monogenic human diseases (cardiovascular diseases, nephrological diseases, recurrent spontaneous abortions).



Professor Petersen studied Medicine at University of Copenhagen, Denmark (1983), and subsequently specialized in Clinical Genetics (Danish National Board of Health, 1997). He has a PhD (1992) and DMSc (1996) from University of Copenhagen, on linkage mapping of human chromosome 21 and origin of

nondisjunction in trisomy 21, respectively. He did a postdoctoral Fulbright fellowship in Molecular Genetics at the Center for Medical Genetics, Johns Hopkins University School of Medicine, Baltimore, USA, 1988–1990. From 1993–2012, he directed the Department of Genetics at the Institute of Child Health, Athens, Greece. Since March 2012 he serves as Chief Consultant at the Department of Clinical Genetics, Aalborg Hospital, Denmark. His main research interests have been origin and mechanisms of chromosomal aneuploidies, but he has in the more recent years concentrated on genetics of deafness and blindness. He has published more than 170 articles in peer-reviewed international genetics journals, and he is an Editorial Board member of four international genetics journals. In 2004, he was awarded a Distinguished Visiting Professorship at the "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania.



George Kitsos is Associate Professor of Ophthalmology in the Ophthalmology Clinic of the Medical School of University of Ioannina, Ioannina, Greece. He received his medical degree from the University of Thessaloniki, Greece and his PhD from the Medical School of the University of Ioannina, Greece. He was also guest researcher in Centre Hospitalier National d'Ophtalmologie des Quinze-

Vingts, Paris, France. He has been an invited speaker and chairman in numerous international conferences. He has authored over 55 publications which received over 370 citations. His main scientific interest is the field of glaucoma as well as genetics and neuro-ophthalmology.