Review

Biomarkers in primary open angle glaucoma

Haris Kokotas¹,²,*, Christos Kroupis³, Dimitrios Chiras⁴, Maria Grigoriadou¹, Klea Lammissou², Michael B. Petersen⁵ and George Kitsos⁶

¹ Department of Genetics, Institute of Child Health, ‘Aghia Sophia’ Children’s Hospital, Athens, Greece
² Department of Genetics and Biotechnology, Faculty of Biology, University of Athens, Athens, Greece
³ Department of Clinical Biochemistry, Attikon Hospital, Athens University Medical School, Athens, Greece
⁴ Department of Ophthalmology, Ioannina University Medical School, Ioannina, Greece

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.

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

*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
ric. It is defined by optic disc or retinal nerve fiber structural disease process that is generally bilateral and often asymmet-
ing from 1.1% (7) to 3.8% (8). POAG is a long-term ocular mon form of glaucoma, with reported prevalence rates rang-
ing than 50 million people worldwide (6) . It is the most com-
itive family history (9) . Since POAG is a chronic condition, it
must be monitored for life, but early diagnosis is the first and
thetic conditions, and, therefore, are frequently used for spe-
ific biomarker selection. Indeed, the most important criterion
logic conditions, and, therefore, are frequently used for spe-
ification (e.g., in case of gene mutations) or quantitatively (e.g.,
protein concentration in urine). Molecules can change qualita-
tively (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/path-
cation. 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 intercellu-
lar 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 qualita-
tively (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/path-
cways attributable to concrete pathologic condition. Generally,
bio markers should not be necessarily linked to any genetic
mutation; both mRNA expression and protein levels possess
valuable information about cellular response to distinct patho-
logic conditions, and, therefore, are frequently used for spe-
cific 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 valu-
able 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 mark-
ers, 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

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 pathogen-
esis 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 asso-
ciation 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 mea-
sured with adequate accuracy and precision and evaluated as
indicators of normal biological or pathogenic processes, or to
monitor pharmacologic responses to a therapeutic interven-
tion. 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 intercellu-
lar 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 qualita-
tively (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/path-
cways attributable to concrete pathologic condition. Generally,
bio markers should not be necessarily linked to any genetic
mutation; both mRNA expression and protein levels possess
valuable information about cellular response to distinct patho-
logic conditions, and, therefore, are frequently used for spe-
cific 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 valu-
able 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 mark-
ers, 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

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 com-
mon form of glaucoma, with reported prevalence rates rang-
ing from 1.1% (7) to 3.8% (8). POAG is a long-term ocular
disease process that is generally bilateral and often asymmet-
ric. 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 sug-
gested to be a neurodegenerative disorder which is triggered
by different factors including mechanical stress due to intra-
ocular 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 appear-
ance 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 abnormali-
ies 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 reduc-
tion in the level of IOP lessens the risk of visual field pro-
gression in open-angle glaucoma. Apart from IOP, other risk
factors include old age, race, female gender, myopia, corneal
thickness, optic disc hemorrhages, hemodynamics, and posi-
tive 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

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
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

3α-HSD

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

Weinstein et al. determined whether peripheral blood lymphocytes from POAG patients have reduced 3α-HSD activity (16). The results of this study showed an association of decreased peripheral blood lymphocytes 3α-HSD activity and POAG which was not related to antiglaucoma therapy. The authors concluded that the reduced levels of 3α-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 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) superfam 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).

AP₃,A

Diadenosine tetraphosphate (AP₃,A) is a compound that contains two adenosine moieties bridged by four phosphates. AP₃,A is found in tears, heart, and brain (40). Oxidative stress induces synthesis of AP₃,A (41). In vivo, AP₃,A 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 juxtasacanalicular 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, pathophysiologic process in brain ischemia as well as in atherosclerotic plaque development (71). In a study by Duan and coworkers, 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,
suggested 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 \( \alpha \) (TNF-\( \alpha \)), 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-\( \alpha \) 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-\( \beta \) (TGF-\( \beta \)) exists in at least five genetically distinct isoforms, \( \beta_1, \beta_2, \beta_3 \), and \( \beta_4 \) are expressed in human ocular tissues (81). TGF-\( \beta_2 \) is regarded as the major isoform in the eye (78, 80, 81). Elevated levels of TGF-\( \beta_2 \) 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 juxtascleralic region of the TM. TGF-\( \beta_2 \) signaling may be involved, as TGF-\( \beta_2 \) is significantly increased in the AH of patients with POAG. In cultured human TM cells, TGF-\( \beta_2 \) causes an increase in extracellular matrix deposition (85). The concentration of TGF-\( \beta_2 \) has been previously measured in several studies (86–88). The results have shown that the levels of total TGF-\( \beta_2 \) in the aqueous samples of POAG are elevated and have suggested that minimizing TGF-\( \beta_2 \) levels may help to prevent the aging process in the TM as seen in POAG (88). Extracellular matrix elasticity modulates TGF-\( \beta \)-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. Sakana 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 endoplasmic reticulum stress transducers and serves as a transmitter in altering endoplasmic reticulum homeostasis. These proteins originally generate a cytoprotective signal leading to reduced translation, then improve endoplasmic reticulum protein folding capacity, and clear misfolded endoplasmic reticulum proteins (96). Studies performed during the last decade identified GRP78 as a ubiquitous luminal resident protein of the endoplasmic 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 endoplasmic 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 endoplasmic reticulum stress inducer (97).

**Heparin**

Heparin is a small peptide produced in the liver. Human Heparin is produced from an 84 amino acid precursor, including a putative single peptide. Heparin 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 Heparin is expressed in Muller cells, photoreceptor cells, and retinal pigmented epithelium. The expression of Heparin in the retina points to the local intraocular regulation of iron metabolism, separate on a dependence of the circulation liver-derived hormone: circulating Heparin 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 methionine-homocysteine 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 amino 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 μmol) 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 in 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 (nNOS; 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).

Transferrin

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 disperse 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).
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.
References


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.


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.