REVIEW

RJME
Romanian Journal of Morphology & Embryology
http://www.rjme.ro/

The morphogen behind primary congenital glaucoma and the dream of targeting

KYRILLUS SAMAAN SHOHDY[1)], WEGDAN ALY RASHAD[1)], MOHAMED KHALID FARGOUN[1)], PHILLIPPE URBAN[2)]

[1)]*Faculty of Medicine, Cairo University, Cairo, Egypt*
[2)]*CNRS UMR5504, Laboratoire d'Ingénierie des Systèmes Biologiques et des Procédés, Université de Toulouse, Toulouse, France*

Abstract

Glaucoma is a clinical entity with multifactorial etiology, a severe subtype occurs in infancy called primary congenital glaucoma (PCG). Three distinct levels interact sequentially to produce PCG: (*i*) genetic mutations mainly affecting the *CYP1B1* gene, (*ii*) absence or dysregulation of a morphogen, and (*iii*) trabecular meshwork pathological changes either in patterning or remodeling. We will discuss these three levels in detail towards further understanding of the morphological basis of the disease, focusing on the missing points, for instance the exact nature and function of the morphogen along with the putative role of *CYP1B1* gene.

Keywords: congenital glaucoma, genetics, *CYP1B1*, morphogen, retinoic acid, periostin.

Introduction

It is intriguing that a single mechanical defect can cause all these deleterious ramifications in such a complex structure – our eye. A gene defect (or possibly more) results in harmful metabolite production or absence of a pattern-forming molecule, *i.e.*, morphogen. This metabolite causes damage that with time becomes irreversible. Eventually, the trabecular meshwork (TM), the main drainage system of the anterior chamber, becomes dysfunctional where unknown developmental defect(s) occur (trabeculodysgenesis). The drainage process is impaired, the aqueous humor accumulates, the intraocular pressure increases and the weakest point of the eye afflicted by the high pressure is the posterior segment, namely the optic nerve and the ganglion cell layer in the retina. Eventually, optic neuropathy and visual impairment ensues; the former explains our current understanding of the pathogenesis of primary congenital glaucoma (PCG). Recently, PCG has been considered a panocular disorder; the entire ophthalmic structure will be affected at some point of the disease course due to the progressive globe distention. The following is the most notably encountered altered corneal biomechanics, iris stromal hypoplasia, *ectopia lentis*, and Descemet membrane breaks, also rhegmatogenous retinal detachment is reported as a late complication [1–3].

Ultrastructure of trabecular meshwork

The drainage system of anterior chamber is formed from three main structures; the trabecular meshwork, Schlemm's canal, and finally intrascleral and episcleral venous plexi. TM is a sponge-like structure where collagen fibers form scaffolds in shape of beams and plates lined exteriorly with endothelial-like cells, *i.e.*, trabecular cells. TM is further organized into three differentiated layers (Figure 1), which are from the innermost: (*i*) uveal meshwork, formed of several irregular layers in shape of prolongations or beams of extracellular matrix (ECM) which is entirely covered by endothelial cells, *i.e.*, TM beam cells (Figure 1a) – these prolongations appeared to be continuous with that of the iris and ciliary body stroma; (*ii*) corneoscleral meshwork, the beams become more flattened to form lamellae or sheets, which are also covered totally with TM monolayer, the center of the lamellae is rich in collagen fibrils and elastic fibers, this layer is highly organized compared to the uveal layer with narrower intercellular spaces and this poses more resistance to the flow of aqueous humor (AH) (Figure 1b) [4]; (*iii*) juxtacanalicular meshwork (JCM), the architecture is altered; instead of ECM covered with cells in the previous two layers, the cells of this layer are embedded in a dense amorphous ECM, also some JCM cells are scattered on the trabecular surface hence the name cribriform meshwork – this layer is in direct contact with the inner wall of Schlemm's canal (Figure 1c) [5]. Another peculiar character of JCM cells is that they lack the basal lamina, and considered to be fibroblast-like, however some studies could identify basement membrane protein nearby the JCM cells. The special architecture of JCM, being filled with ECM gel, makes it the major source of flow resistance in the human eye [6]. Once the JCM cells and ECM undergo mechanical stretch from elevations of intraocular pressure (IOP), and sense this stretch *via* integrins and other receptors, they respond by two mechanisms: (*i*) modulating the synthesis of a wide variety of extracellular and intracellular genes, and (*ii*) increasing proteolytic enzyme activities, *e.g.*, matrix metalloproteinases-2 and -14 with concomitant decrease of their inhibitors, *e.g.*, tissue inhibitor of metalloproteinase-2 (TIMP-2) [7].

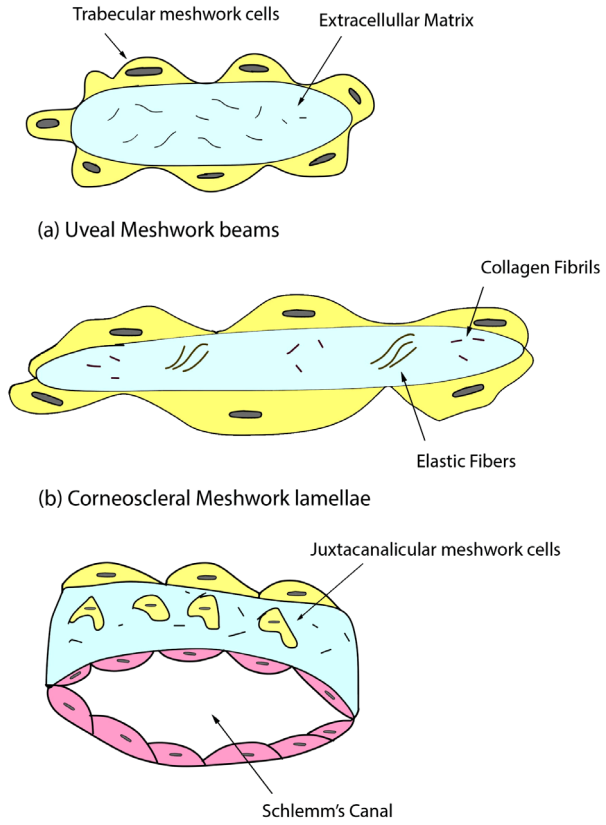

Figure 1 – ***The structural units in each of the three differentiated layers of the trabecular meshwork.***

Development of trabecular meshwork

The cascade of development of TM entails three main steps:

(1) Migration

Mesenchymal cells start to migrate to the anterior margin of the developing optic cup; these cells are called periocular mesenchyme (POM). The origin of POM turned out to be mainly from cranial neural crest cells (NCCs) and to minor extent from paraxial mesoderm. The migration occurs in three waves, the second wave resides at the angle between the anterior edge of the optic cup and the cornea, and these cells differentiate into the stroma of iris and ciliary body.

(2) Differentiation and patterning

The mass of POM cell, occupies the future TM in the angular recess between the cornea and the iris, start to differentiate into channels and form the TM. In regard to how the intertrabecular spaces appeared in this bulky mass of tissue is still elusive. Previously, it was postulated that atrophy and cell death is responsible for forming these spaces. However, Smith *et al.* [8] have found that cell death and macrophages were not detectable during the various developmental stages of TM.

(3) Remodeling

As the recent models of TM development have excluded the atrophy and cell death instead, organization of cellular and extracellular matrix components do occur. In mice, the TM beams start to separate and ECM becomes deposited at postnatal day 10 (P10). By P14, the spaces between TM beams are well established. The intertrabecular spaces continue to enlarge by the end of P18 [8].

Pathological changes in PCG

The pathology of PCG is not clear as most available specimens from enucleated human eyes show severe and more complicated pathologies, which obscure the primary one. This limitation in human pathological specimens along with the notion that *CYP1B1* either produces or transforms the morphogen behind TM development, has urged Teixeira *et al.* [9] to depend on *CYP1B1* deficient mice to study the abnormalities of TM *via* histological examination of postnatal iridocorneal angle of *CYP1B1*–/– mice. They have found that TM is more or less normal during first and second postnatal week and the disruption of TM architecture starts at the third week (correlates with final stages of TM development in mice), where the trabecular beams atrophied and intertrabecular spaces accentuated, until there is only one atrophied trabecular beam left, with eventual collapse of TM. This study excluded that the underlying pathology caused by absence of *CYP1B1* is due to cell migration failure during embryogenesis rather, failure of ECM deposition or ECM remodeling and the latter is more likely. Another earlier study [10] from the same group has stressed the role of periostin (Postn) in TM remodeling and development, they have found that in the CYP1B1–/– mice and the glaucomatous human eyes, the Postn level was decreased. The previous study sheds light on importance of ECM of TM in pathogenesis of PCG; interestingly, the cellular component of TM is incriminated in primary open angle glaucoma, it also added more on the 2003 study by Libby *et al.* [11]. However, the latter study assessed the anterior chamber developmental defects in tyrosinase deficient mice and could find that L-DOPA plays an important role in anterior chamber development. The mice, which are CYP1B1 and tyrosinase deficient, showed more severe angle dysgenesis than CYP1B1-deficient only mice. Moreover, these dysgenetic changes could be corrected to some degree with L-DOPA intake with drinking water. An interesting molecular model proposed by Libby *et al.* [11] demonstrated that retinoic acid (RA) enhances proliferation of avian neural crest cells from which the TM and iris stroma is derived and those cells express tyrosine hydroxylase (TH) (which converts tyrosine to L-DOPA), so the defect in CYP1B1 aborts RA biosynthesis and the absence of the latter lead to under-proliferation of neural crest cells, hence decreasing TH and L-DOPA. This model lent weight to the hypothesis that we will later discuss about RA being a putative morphogen behind PCG.

The recent study from Zhao *et al.* research group [10] may be interpreted that PCG is a problem of remodeling not patterning. However, the hunt for the morphogen or substrate of CYP1B1 is still the crucial step in understanding the pathogenesis of PCG in regard to the other important substances that are proposed now for disruption of the remodeling step like Postn. They believe this is influenced by the richer oxygen environment during postnatal life and it is the accumulation of oxidative stress that drives the dysfunction of developing TM due to accumulation of its substrate (Sheibani N, personal communication). This also can be correlated with that

Postn is a key modulator of pressure-induced homeostatic response and its down regulation may expose the TM to a vicious circle of IOP elevation that cannot be interrupted. It would not be a wild speculation if we suppose that PCG is caused not due to developmental defect occurring during migration, differentiation or patterning of TM rather than being due to loss of protective regulatory mechanism(s) in TM cells that Postn shares in part in one of them. This is supported by recent studies that have revealed the role of *CYP1B1* mutations in primary open angle glaucoma (POAG) and juvenile open angle glaucoma (JOAG) [12]. *CYP1B1* mutations seem to be implicated in a wide spectrum of glaucoma phenotypes, reviewed by Choudhary *et al.* [13]. This is correlated to two distinct yet interrelated functions of this enzyme. The first function is developmental role in the differentiation or remodeling of trabecular meshwork and other anterior chamber structures where the reduction or abundance of enzymatic activity [14] leads to three different anterior segment dysgenesis phenotypes, which are PCG, Peter's anomaly and Axenfeld–Rieger's syndrome [15–17]. The second function is the homeostatic regulatory response to the change in IOP where the enzymatic activity produces substance(s) that modulate the ECM and scavenge harmful oxidants and disturbance of this regulatory response share in development of POAG and JOAG. Vincent *et al.* have found that the co-existence of mutations in myocilin and *CYP1B1* accelerate the onset of JOAG in earlier age [12].

Genetics of PCG

It is apparent that PCG (gene symbol, *GLC3*) is essentially a genetic disorder with a multitude of gene defects, in the 1980s this genetic heterogeneity of PCG was postulated [18] and since then numerous studies continued unraveling the etiopathogenesis of the severest form of glaucoma in infancy (Table 1). Sadly, there has been minimal development of medical therapeutics as alternatives to surgical treatment, which remains the mainstay in management options. To the best of our knowledge, there are four candidate loci linked to such condition (Table 2). However, the GLC3A locus, which expresses a cytochrome P450 protein called CYP1B1 is implicated in the majority of cases. *CYP1B1* gene belongs to the superfamily of cytochrome P450 and has its locus on the short arm of chromosome 2 (2p21), this gene was first reported by Sarfarazi *et al.* [19] to be a major gene in the pathogenesis of PCG.

A founder effect is evident in Gypsies (Slovak Roma) and Middle Eastern populations where the highest prevalence is reported, 1:1250 and 1:2500, respectively [20]. However, around 147 different mutations from different populations are identified in this gene including missense, non-sense, frameshift, deletion, and insertion or duplication mutations [21], of course this number increases every year with recent studies on different cohorts of patients [22, 23]. *CYP1B1* mutations account for 80% of familial and 33% of sporadic PCG cases, not to mention that in some subpopulations (Saudi Arabians and Slovak Gypsies) they account for 100% of the cases [24]. The missense mutations constitute the majority of *CYP1B1* mutations around 39% (n=214 cases in 14 studies recruiting 550 cases according to Kaur *et al.* [25]).

Table 1 – ***Milestones in genetics of PCG***

Year		Reference
1950	Waardenburg claimed a recessive inheritance pattern in some glaucoma cases.	[26]
1976	Briard *et al.* postulated a clinical and genetic heterogeneity in category of nonsyndromal or primary congenital glaucoma.	[27]
1980–1982	Genetic heterogeneity and recessive inheritance of PCG were much supported with works of Genčík *et al.*, Demenais *et al.* and Morton.	[18, 28, 29]
1995	2p21 assigned as candidate locus for PCG (in 11 families out of 17).	[19]
1996	6p21 and 11p15::q12 excluded as candidate *loci* for PCG.	[30]
1996	1p36 assigned as second locus for PCG.	[31]
1998–2000	Mutations of cytochrome P450 1b1 on 2p21 is implicated as principal cause of PCG.	[32, 33]
2002	14q24.3 assigned as third locus for PCG.	[34]
2009	Null mutations in *LTBP2* gene cause PCG in four consanguineous Pakistani families and in eight out of 15 CYP1B1-negative Gypsy patients.	[35]

PCG: Primary congenital glaucoma; *LTBP2*: Latent transforming growth factor (TGF)-beta binding protein 2.

It is noteworthy that *CYP1B1* gene mutations can lead to another very rare condition called Peters' anomaly, which entails central corneal opacity and corneal adhesions to the iris, with or without adhesions to the lens [36, 37] This reinforces the point of view about the role of *CYP1B1* in directing the development of the anterior segment.

In some countries where the genetic studies are readily conducted on PCG patients and pedigrees, the frequency of a certain gene mutation is measured and the common ones are used for screening purposes for example in the Japanese population the following three mutations appear to be common: p.Asp192Val, c.4776insAT, and p.Val364Met [15], in Morocco, the g.4340delG mutation seems to be predominant [38], whereas the p.Gly61Glu mutation predominates in Saudi Arabia [39].

The recent evidence is against that mutations in the latent transforming growth factor (TGF)-beta binding protein 2 (*LTBP2*) gene can lead to PCG (isolated trabeculodysgenesis) rather they cause more complex anterior segment developmental defects, *e.g.*, microspherophakia, *ectopia lentis* and megalocornea, where glaucoma develops secondarily [24, 40]. Though the *LTBP2* gene location is around 1.3 Mb proximal to GLC3C locus, it is unlikely that *LTBP2* is the *GLC3C* gene and perhaps a second adjacent gene is implicated [35, 41]. One recent study has recruited 54 unrelated patients with PCG compared to 50 normal controls, they were either negative or heterozygous for MYOC, CYP1B1, and FOXC1 mutations, yet no pathogenic variants were identified in the *LTBP2* gene in those patients [42]. Furthermore, the localization of LTBP2 protein in experimental animal models revealed abundant immunoreactivity in the ciliary body with the highest intensity in the ECM of ciliary processes [35]. This suggests that LTBP2 shares in the maintenance of shape and elasticity of the ciliary body,

which is important in supporting the TM and in homeostasis of AH drainage.

So, determining gene *loci* of *GLC3B*, *GLC3C*, *GLC3D* is a much needed task for more understanding of the heterogeneity principle of PCG and the implicated genes beside the largely documented CYP1B1, of these genes, *LTBP2* (MIM #602091), *MYOC* (MIM #601652) and *FOXC1* (MIM #601090) [23].

Table 2 – ***Gene loci of primary congenital glaucoma***

Candidate locus	Candidate gene	Mapping on chromosome	Reference
GLC3A	*CYP1B1*	2p21 [MIM #231300]	[19]
GLC3B	N/A	1p36 [MIM #600975]	[31]
GLC3C (613085)	N/A	14q24.3-q31.1	[34]
GLC3D (OMIM 613086)	*LTBP2* (controversial)	14q24 [MIM #602091] (outside GLC3C locus)	[35, 43]

N/A: Not available; *LTBP2*: Latent transforming growth factor (TGF)-beta binding protein 2; OMIM: Online Mendelian inheritance in man; MIM: Multiple-interval mapping.

The enigmatic CYP1B1 and the eye

CYP1B1 is a monooxygenase acting on both xenobiotics and endobiotics, the CYP1B1 mRNA and protein were found to be expressed in a variety of normal human tissues [44], and its cellular localization showed to be nuclear in the majority of examined samples with two exceptions; the renal tubular cells and the mammary gland secretory cells where the immunoreactivity of the protein was intense in both the nucleus and the cytoplasm. Moreover, one recent study [45] could specify the location of CYP1B1 protein to mitochondria as well as to endoplasmic reticulum. There is much investigation into the actions of CYP1B1 protein on xenobiotics rather than endobiotics as it is linked to pathogenesis of many types of tumors namely breast and lung cancers [46, 47].

There is a paucity of studies about the functions of CYP1B1 in the eye; the recent hypothesis is that CYP1B1 protein shares in the development of the anterior segment of human eye in the fetal period either through a substrate or a metabolite produced from its enzymatic action. This assumed substrate(s) or metabolite(s) (termed morphogen) is not determined precisely. However, according to comparative studies between the human and mouse orthologues of the same gene, some endobiotics could be ruled out, *e.g.*, arachidonic acid and its metabolites, *β*-estradiol, testosterone and progesterone, while others still bear the possibility to be that morphogen, *e.g.*, retinoids [13, 48]. Actually, it is tempting to speculate that the morphogen may in fact be retinoic acid (RA) on basis that CYP1B1 shares in synthesis (not degradation) of RA [both all-*trans*-retinal (t-RAL) and all-*trans*-retinoic acid (t-RA)] from retinol (Figure 2, a and c), and the former being a ligand for different nuclear receptor proteins, has a regulatory function in anterior segment morphogenesis *via* modulating gene transcription [49]. In POM of the mouse eyes, RA signaling is active from embryonic day 10.25 (E10.25) [50].

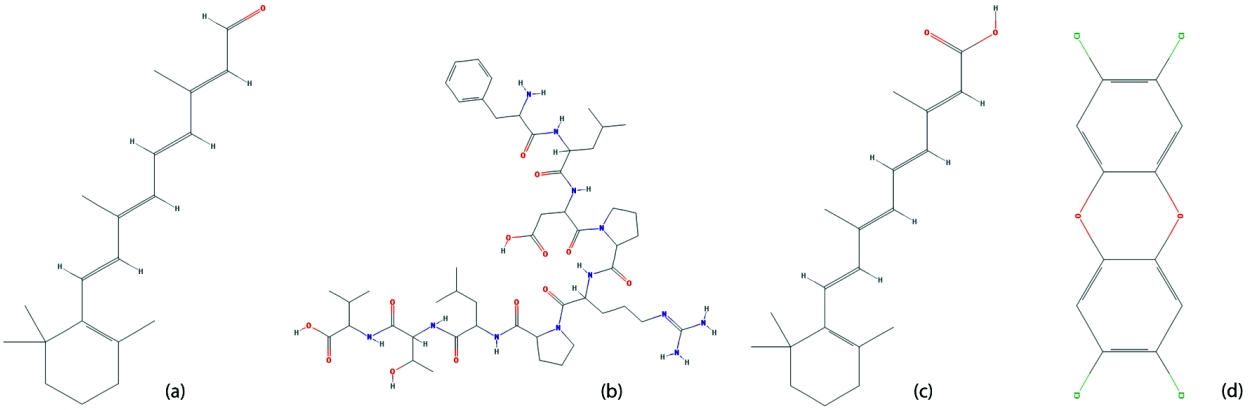

Figure 2 – ***Chemical structures of some relevant compounds to primary congenital glaucoma: (a) All-trans-retinal; (b) 2D structure of CYP1B1 in Homo sapiens (190–198); (c) Retinoic acid; (d) 2,3,7,8-Tetrachlorodibenzo-p-dioxin.***

Moreover, RA is a well-known endogenous morphogen in limb development; it has been found that RA is continuously released by zone of polarizing activity (ZPA), which is a region of mesenchyme at the posterior margin of the limb bud [51]. ZPA secretes RA in a graded distribution to direct the patterning and specification of the various limb structures.

We cannot rule out totally the possibility that steroids or arachidonic acid metabolites share in signaling system important for morphogenesis of TM. A lipid receptor signaling system based on G protein-coupled receptor 18 (GPR18) was recently investigated for regulation of IOP [52]. Moreover, some morphogens are prone to lipid-modification and this controls their transport and their diffusivity.

This is further supported by the notion that considers anterior chamber as a specialized blood vessel, the AH is the blood and the endothelial-like cells of TM along with corneal endothelium are the vascular wall [53]. Hence, TM is subjected to the same biological parameters of vascular wall. It is known that metabolites of arachidonic acid and polyunsaturated fatty acid products have a key role in modulating of vascular tone, blood flow and angiogenesis [54]. Interestingly, one study revealed that *CYP1B1* gene and protein expression in endothelial cells of mouse blood vessels is regulated by shear stress [55]. Similar study to be conducted on TM cells will be of great value. Further research on the role of CYP1B1 in fat metabolism is needed along with the revelation of other lipid-based signaling systems in anterior chamber. It is tempting

that this research shall start with the G protein-coupled receptors (GPCRs). GPCRs are the most famous cellular receptors that bind fatty acids and downstream a versatile signaling systems. For example, GPR48 knockout mice show a wide spectrum of anterior segment dysgenesis (ASD) including microphthalmia, iris hypoplasia and iridocorneal angle malformation [56]. GPR48 (also known as LGR4) is an orphan receptor that mediates cyclic adenosine monophosphate–protein kinase A (cAMP–PKA) and CREB (cAMP responsive element binding protein) signaling pathway (Figure 3).

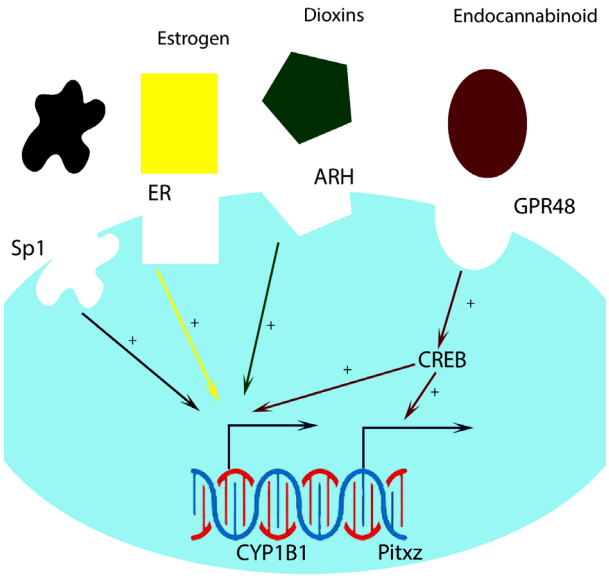

Figure 3 – ***Regulation of CYP1B1 expression via ARH-dependent pathway with exogenous ligands such as TCDD and via ARH-independent pathways including transcription factors such as Sp1, CREB and ER. ARH: Aryl hydrocarbon receptor; TCDD: 2,3,7,8-Tetrachlorodibenzo-p-dioxin; CREB: cAMP (cyclic adenosine monophosphate) responsive element binding protein; ER: Estrogen receptor; +: Activate.***

CREB, a transcription factor that binds Pitx2 exactly at the CRE-binding site of the *PITX2* promoter thus *GPR48* is capable of regulating *PITX2* [56]. Actually, *CYP1B1* is known to cross talk with this pathway, as CREB is one of the transcription factors that regulate expression of *CYP1B1* [57]. Other examples are the *GPR84* and *GPR161* that are implicated in lens and retina development [58, 59]. Besides the developmental role of the orphan *GPR48*, *84*, *161* [60], the *GPR18*, a deorphanized lipid receptor, found to have a functional role in IOP regulation [52]. Interestingly, *GPR18* is acted upon by the endogenous lipid *N*-arachidonoyl glycine (NAGly) and the abnormal cannabidiol (Abn-CBD).

To sum it up, CYP1B1 protein is involved in the metabolism of steroids, retinol and retinal, arachidonate, and melatonin. The conserved expression of CYP1B1 in all vertebrates, its higher expression in fetal than adult eyes, and its biochemical properties are consistent with this hypothesis. The exact role of CYP1B1 in the pathogenesis of glaucoma and other ASD disorders remains to be elucidated.

The characterization of expressed proteins that lead the proper development of TM is still a dark area (Table 3), as the majority of PCG cases are linked to *CYP1B1*, which expresses a 543 amino acid long protein. CYP1B1 is formed from (*i*) 53 residue long *N*-terminal region, which is bound to the endoplasmic membrane, (*ii*) the hinge area, formed of 10 amino acids and rich in proline, (*iii*) 480 residue long cytosolic globular domain. The crystal structure was elucidated by Wang *et al.* (Figure 4) [61]. The crystallographic study of CYP1B1 revealed a high conservation of around 18 out of 20 amino acid residues in the active site across the vertebrate species. Most active site cavities of P450s show extensive structural and functional diversity due to gain and loss of genes during evolution. Meanwhile, CYP1B1 has kept a very constant slot-like active site cavity that is encoded by single gene in most vertebrate genomes [61]. This cavity is well adapted for binding hydrophobic planar bulky aromatic systems, *e.g.*, polycyclic aromatic hydrocarbons, flavonoids and retinoids.

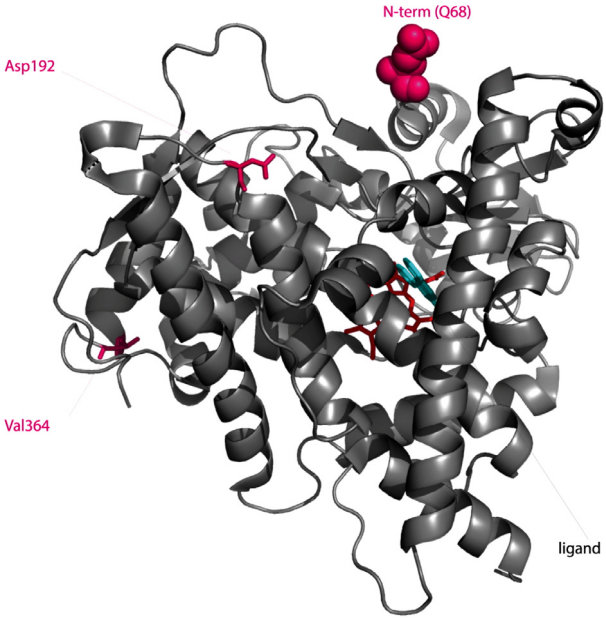

Figure 4 – ***Position of the different PCG mutations shown in the light of the human CYP1B1 crystal structure. The Val364 mutation is close or in the helix J, that is supposed to be at the interface of the P450-CPR complex. (View of the distal side, with the heme in red sticks and the ligand: alpha-naphthoflavone in sticks colored cyan, PDB entry: 3pm0). The N-terminus of the crystal structure is Gln68 because the crystallized CYP1B1 misses the 67 first amino acid residues for sake of solubility. PCG: Primary congenital glaucoma; CPR: Cytochrome P450 reductase; PDB: Protein Data Bank.***

The search for the expressed protein of CYP1B1 in the TM tissues in either murine or human eyes has failed to detect anything significant until the study of Zhao *et al.* [10]. The contradictory results, where some could detect CYP1B1 protein in TM and others could not, left us with two hypotheses about the nature and function of PCG morphogen. The first, with more evidence (Table 3) being that the morphogen is expressed in ciliary body cells, which secrete the aqueous humor and it flows to TM, to exert its function there, this hypothesis can be termed *flowing morphogen*. Actually, CYP1B1 is first detected in primitive ciliary epithelium at embryonic day 26 (E26) in humans even before the TM starts to develop and

it shows concentration profile changes parallel to the developmental changes in the TM [13, 62].

The second hypothesis is that the morphogen is expressed in TM cells themselves and exert an antioxidant effect as well as increasing the levels of Postn as proposed by Zhao *et al.* [10]; this hypothesis can be termed *in situ morphogen* although the term *morphogen* will not be correct in this situation. This is supported by the notion that the pathological changes in PCG cases indicate a problem in remodeling not patterning (see the section: "Pathological changes in PCG"). It is noteworthy that Postn itself has no structural role, but it binds ECM proteins and could modulate the cell phenotype and function. This matricellular protein is known to be up-regulated in two conditions; tissue injury and remodeling tissues [63]. The role of Postn in remodeling of TM is still elusive.

Further studies are crucial to settle these two points of view or perhaps reconcile them in one big hypothesis explaining the correct pathogenesis of PCG and paving the road for therapeutic targeting as the conventional medical treatment continues to show disappointing results: *e.g.*, in one recent study on long-term efficacy of latanoprost (prostaglandin F2a), it was found to be effective in about 30% of the eyes, without prior surgery [64].

In conclusion, we can point out two main expected functions of CYP1B1 protein that need to be investigated (*i*) removing a metabolite which is toxic to TM cells, (*ii*) inducing generation of a molecule that regulates other genes involved in the anterior segment development.

Table 3 – ***The studies conducted to detect expression and distribution of CYP1B1 in ocular tissues***

Detection	Methodology	Species	Type of ocular tissues		Reference
			Present in:	*Absent in:*	
CYP1B1 mRNA expression	*In situ* hybridization	Mouse eyes	Pigmented ciliary epithelium and sensory retinal neuroepithelium	Trabecular meshwork and iris	[65]
CYP1B1 protein	Indirect immunofluorescence	Human and mouse eyes	Primitive ciliary epithelium, non-pigmented (inner) ciliary epithelium (ICE), corneal epithelium, iris, retina.	Trabecular meshwork	[66]
CYP1B1 protein	Immunohistochemistry	Adult mouse eye and newborn	ICE, corneal epithelium, retinal inner nuclear cells, and ganglion cells	Trabecular meshwork	[62]
CYP1B1 protein	Immunohistochemistry	Human and mouse eyes	Trabecular meshwork		[10, 67]

Toward a morphogen gradient system for TM

The classical morphogen gradient system entails a source of production, a transport mechanism and a receptor that downstreams a signaling pathway. Most morphogens are secreted polypeptides that bind cellular receptors that mediate signaling and remove the morphogen itself from the developmental field [68].

The theoretical work proposed by Turing [69], Wolpert [70] and Crick [71], including positional information and source-sink hypothesis, can be applied on RA as a morphogen; a dynamic concentration gradient is created in cell lines responsible for development of anterior chamber, on one pole of this cell line (which may be the future ciliary body) the CYP1B1 is produced abundantly, which in turn synthesizes large amount of RA. On the other side of the pole (the future TM), the RA gets degraded, a concentration gradient is generated, and this gradient directs the development of TM and that could explain why most of studies fail to detect significant level of CYP1B1 protein in TM cells. PCG is like a mutilated corpse in a crime scene, *i.e.*, TM without any weapon in the vicinity so full inspection of the eye structures is needed. This is supported with the role of RA in limb morphogenesis as discussed before. Another important source of RA is the retina, which expresses retinal dehydrogenases 1 and 3 (RALDH1 and RALDH3); both enzymes synthesize RA, which in turn diffuse to periocular mesenchyme in a paracrine manner. The RALDH deficient zebrafish produced anterior segment dysgenesis most notably cornea and eyelid, there was no affection of the TM [72]. This indicates that there is RALDH-independent generation of RA, which enables embryos to direct the RA-mediated patterning. The CYP1B1 and RALDHs share a complementary role in the production of RA (Figure 5) [73].

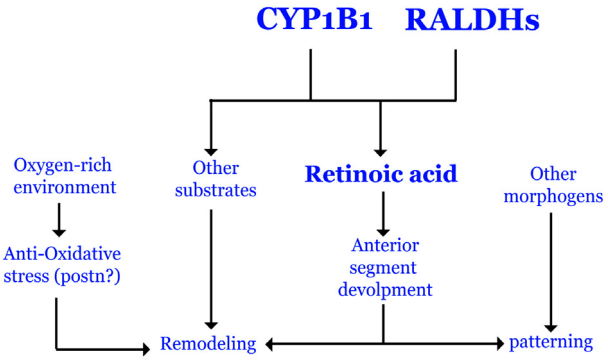

Figure 5 – ***Flowchart indicates factors implicated in TM development and PCG pathogenesis. TM: Trabecular meshwork; PCG: Primary congenital glaucoma; Postn: Periostin; RALDHs: Retinal dehydrogenases.***

One study in zebrafish embryos has shown that there is a specific spatial and temporal pattern of CYP1B1 expression in the dorsal and ventral retina. An overlap between CYP1B1 and RALDH2 and 3 were also evident [74]. The RA-degrading enzymes (CYP26A1,C1) have been found to be expressed in a narrow area flanked by the expression boundaries of RALDH1 and RALDH3 in the retina [75].

The concentration gradient of RA is produced in variable patterns, where in a specific spatio-temporal dimension it is generated by RALDHs and in another point of time, it is produced or modulated by CYP1B1. CYP1B1 may just modulate the RA dosage at some point of time, the very same critical dose needed for TM to complete its growth and modeling. CYP1B1-generated

RA amount is added on a basal amount already having been produced from RALDHs and diffuse constantly to the TM region.

Let us imagine that the morphogen produced from three areas; dorsal, ventral retinae and ciliary body, is transported towards the iridocorneal angle and it is not unlikely that the angle itself becomes a fourth area of morphogen production. Every area contributes to the production of the morphogen(s) of the anterior chamber at the same rate, it is logical that what is produced from the posterior points, *i.e.*, dorsal and ventral retina will get degraded more rapidly than what is produced anteriorly from CB or TM. Hence, the cells of the anterior segment are subjected to different concentrations in a steady-state condition. The threshold responses for every population of cells in the anterior segment is different, those cells that will form the cornea, iris and eyelid respond at lower threshold and those that form the TM respond at higher threshold. The cell population that forms the TM needs an exact dose of the morphogen whose rate of production is affected by CYP1B1 mutations.

This is not a strict process sometimes, CYP1B1 mutations lead to Peter's anomaly rather than isolated trabeculodysgenesis [76]. This hypothesis can be tested in developing eye models by introduction of a RA-binding protein at escalating doses. At a certain level, the RA-binding protein will deprive the cell population with higher threshold from RA and will not affect those responding to a lower threshold. With meticulous calculation of the amount of RA that is technically removed from the gradient system and correlation with the developmental defects that can be observed, many of the PCG mysteries will be resolved. Of course, this experiment is still valid for any other suggested morphogen with a well-known binding carrier and kinetics profile.

This may explain why deficiency of RALDHs leads to excessive POM growth and a wider spectrum of anterior segment dysgenesis along with dysgenesis of cornea and eyelid sparing the TM [72]. Meanwhile, CYP1B1 deficiency leads to isolated TM dysgenesis. It is noteworthy that the NCCs, when specified express WNT1, RA is known to prevent excessive WNT signaling in POM *via* induction of Pitx2 and DKK2 (Dickkopf WNT signaling pathway inhibitor 2) [77]. This in turn, prevents excessive POM growth and ASD. It is likely that patterning defects result in ASD rather than an isolated TM dysgenesis because they tend to be produced from ectopic concentration gradient in the developmental field [78]. The imbalance between the activator WNT and the inhibitor DKK2 and Pitx2 may be implicated in those patterning defects.

RA trafficking

One of the main pillars for the robustness of the morphogen gradients model is identifying the mechanism of morphogen transport, which may be by active diffusion, free diffusion or restricted extracellular diffusion [78]. An interesting study conducted by Bouhenni *et al.* [79] provided much weight on the importance of RA in anterior chamber development, they analyzed the proteomes of aqueous humor in seven PCG cases (compared to four with cataract, as control), the following proteins were detected at significantly higher levels in PCG cases: (*i*) apolipoprotein A-IV (APOA-IV) – it is a plasma protein responsible for lipid absorption and transport; (*ii*) albumin – may increase due to oxidative stress or another unknown cause; (*iii*) antithrombin 3 (ANT 3) – a protease inhibitor that is expressed in TM and found normally in AH. On the other hand, the following proteins were detected at significantly lower levels than that of controls: (*i*) transthyretin (TTR) – it transports retinol and thyroxin (T4) interestingly it was found at higher levels in POAG; (*ii*) prostaglandin-H2 D-isomerase (PTGDS) – catalyzes the conversion of prostaglandin H2 (PGH2) to prostaglandin D2 (PGD2), the latter is involved in smooth muscle contraction/relaxation, PTGDS binds retinal, RA and other small non-substrate lipophilic molecules; (*iii*) opticin (OPT) – it binds to growth hormone in the vitreous during embryonic retinal development; (*iv*) inter-photoreceptor retinoid binding protein (IRBP) – it binds to retinoids and fatty acids.

Decreased TTR, PTGDS and IRBP levels may hinder RA transport in the AH but will they be significant enough to disturb the TM development? Further investigations are needed to resolve such a dilemma. It is noteworthy that the free diffusion of RA, being a lipophilic molecule, will be limited in the aqueous solutions, so the trafficking of RA entails extracellular transport by TTR, PTGDS and IRBP and intracellular transport by cellular RA-binding proteins (CRABPs) [80].

The response of TM to IOP elevation

Once the IOP is elevated the trabecular meshwork with its main two components, cellular and extracellular matrix is exposed to much compression. The cellular component constitutes the main impedance to AH flow, while the porous fibrous ECM is continuously filled and dilated with the percolated AH, the dilated ECM increases the spaces between cells on the trabecular surface. Actually, we can think of these cells to be serenely floating on the surface of the fluid phase (ECM imbibed with AH) but with elevation of IOP, these cells are compressed and the matrix in turn becomes compacted and flattened, hence the intercellular spaces are narrowed and the permeability decreases [6]. Eventually, this leads to a reduction of AH drainage and further IOP elevation. Of course, at some point the body starts to interrupt this cycle with production of modulating substances that facilitate outflow. Borrás [81] has found three important proteins, which are normally upregulated during pressure-induced homeostatic response of TM, which are periostin, matrix metallopeptidase 2 and matrix Gla-protein (MGP).

For a while, cell loss can be seen as a valid solution for annihilating the impedance to AH flow, however this is not true as the TM cells play a key regulatory role in AH drainage process. They respond to pressure changes and stretch, produce and modulate ECM. Critical cellular loss leads to atrophy of trabecular beams and collapse of the elegant architecture of TM along with its sophisticated regulatory mechanisms. Thanks to the patience of Grierson & Howes, who conducted valuable TM cells counting studies [82]. Similar study correlates between the changes of IOP and cell number in TM will be of interesting value. It is noteworthy that cell loss is different among TM cell subtypes and tend to be higher in the uveal meshwork [83].

Limitations of non-human models

An obvious distinction is currently made between evolution of genes and evolution of form, to make this point clear we will hold a comparison about a loss of function due to a mutation in the *CYP1B1* gene, in both human and mouse eyes. The ultrastructure of TM revealed the same developmental abnormalities, while those abnormalities guarantee developing glaucoma in human eye that does not happen in the case of mouse eyes, this urges some authors to suggest a modifier gene (tyrosinase gene in mice) that aggravates the angle abnormalities. It is proposed that there is a link between CYP1B1 and tyrosine hydroxylase (TH), where the retinoids produced from CYP1B1 promote the proliferation of neural crest cells, which POM derived from and express TH. However, Bidinost *et al.* [84] conducted a single nucleotide polymorphism (SNP) analysis in the *TYR* chromosomal region 11q13-q21, and sequencing of the *TYR* gene in a cohort of Saudi Arabian patients with *CYP1B1*-associated PCG phenotype and found out that tyrosinase is not a modifier gene. Another important aspect is that there is a controversy about occurrence of atrophy and cell death as a developmental step in formation of TM in rodents [8]. To complicate matters some studies prove that RA produced by RALDH1 and 3 plays an important role in controlling apoptosis through regulation of Eya2 and Pitx2 [50].

Other non-rodent models, *e.g.*, pig and zebrafish for study of the role of CYP1B1 in PCG are currently investigated [85].

The role of xenobiotics and developmental toxicology

It is intriguing to know that CYP1B1, which is implicated in PCG, was originally cloned as a dioxin-responsive cDNA, is it a paradox that dioxin, which is basically a teratogen, induces the expression of CYP1B1 [86]. It is now evident that normal development of the anterior chamber of the eye depends on *CYP1B1*, being expressed in a strict spatio-temporal pattern and we know that drugs and other xenobiotics can alter cytochrome P450 expression. It is tempting to speculate that aberrant CYP1B1 induction during the embryonic and postnatal development disrupts the normal distribution of morphogen, in other words, PCG can not occur due to a simple gene defect and mere absence of an enzyme and consequent absence of its endobiotic substrate, *i.e.*, the morphogen, other players can be taken in consideration. An interesting study [57] revealed that exposure of zebrafish embryos to TCDD (Figure 2d) before hatching leads to enhancement of CYP1B1 transcription rate. This effect has not been observed after hatching or after blocking aryl hydrocarbon receptor 2 (AHR2), the receptor for TCDD. This indicates that there are two distinct types of 1B1 transcription; constitutive and TCDD-inducible. Furthermore, in *CYP1B1* knockdown models, the developmental toxicity of TCDD could not be eliminated. This means that constitutive transcription of *CYP1B1* is sustained *via* AHR-independent pathways. Dioxin may elicit its toxicity phenotypes through another pathway rather than the conventional TCDD/ARH/CYP1B1 pathway. This is consistent with the notion that regulation of CYP1B1 expression is mediated by ARH-dependent along with ARH-independent pathways (Figure 3). The latter includes transcription factors such as Sp1, CREB and estrogen receptor [87, 88].

Dream of targeting

Speaking about targeting the morphogen will be premature at this stage, researchers could detect the gene loci and candidate genes responsible for such disease by genetic analysis and segregation techniques but they could not come to conclusive results about the encoded protein, *i.e.*, CYP1B1 enzyme and its role in inducing developmental defects in TM, we think that we can benefit from the new techniques investigated nowadays for detection of low abundance proteome [89].

The important question yet to be answered is: will it be too late to develop a targeted therapy that can manipulate the morphogen (which seems to direct the TM development early in embryonic life), even if the diagnosis of PCG is made as early as possible after birth?

The two medical options available now for trial are (*i*) gene therapy: correcting the genetic defects, replacing the pathogenic variants of *CYP1B1*, simply the mutations lead to deficiency of *CYP1B1*, the wild type of *CYP1B1* will be encoded in an appropriate viral vector – this vector is in turn injected in the anterior segment; (*ii*) stem cells: replacing the damaged ganglion cell layer in the retina and corneal endothelium. Meanwhile, the dream of targeting PCG will be too far, how can we attempt to target a molecule that we cannot detect to alter a function that we cannot yet determine?

It is noteworthy that the development of TM is still ongoing postnatally in both human and mouse eyes [13]; there is a chance if effective targeted therapy could be discovered and taken early after birth to save the function of TM and prevent further morbidity.

Conclusions

Our thinking about developmental defects on basis of absence or existence (on/off mechanism) have to be changed to more flexible spatio-temporal changes of induction and inhibition affecting the developing cell lines. The reductionist view of PCG as a genetic defect impairing the development of trabecular meshwork has to be changed to a more integrative combined view that entails: (*i*) migration and patterning defects; (*ii*) defects of the remodeling of extracellular matrix; (*iii*) homeostatic regulatory response for mechanical and oxidative stress; (*iv*) xenobiotics and environmental factors; and (*v*) loss of support from surrounding structures such as ciliary body and lens. The molecular and signaling pathways implicated in the aforementioned points such as tyrosinase/L-DOPA, retinoic acid/WNT signaling, CYP1B1/putative morphogen(s), periostin, dioxin/aryl hydrocarbon receptor and N-cadherin have to be investigated further so that effective targeted therapeutics can be available.

Conflict of interests

The authors declare that they have no conflict of interests.

Acknowledgments

We would like to thank Dr Ahmad Alfaar and Dr Ahmed Awadien for critically reading an early draft of this manuscript.

References

[1] Chang TC, Grajewski AL, Cavuoto KM. Evolving perspectives on congenital glaucoma. Curr Ophthalmol Rep, 2015, 3(2): 85–90.
[2] Patil B, Tandon R, Sharma N, Verma M, Upadhyay AD, Gupta V, Sihota R. Corneal changes in childhood glaucoma. Ophthalmology, 2015, 122(1):87–92.
[3] Gatzioufas Z, Labiris G, Stachs O, Hovakimyan M, Schnaidt A, Viestenz A, Käsmann-Kellner B, Seitz B. Biomechanical profile of the cornea in primary congenital glaucoma. Acta Ophthalmol, 2013, 91(1):e29–e34.
[4] Llobet A, Gasull X, Gual A. Understanding trabecular meshwork physiology: a key to the control of intraocular pressure? News Physiol Sci, 2003, 18(5):205–209.
[5] Acott TS, Kelley MJ. Extracellular matrix in the trabecular meshwork. Exp Eye Res, 2008, 86(4):543–561.
[6] Avtar R, Srivastava R. Modelling aqueous humor outflow through trabecular meshwork. Appl Math Comput, 2007, 189(1):734–745.
[7] Keller KE, Kelley MJ, Acott TS. Extracellular matrix gene alternative splicing by trabecular meshwork cells in response to mechanical stretching. Invest Ophthalmol Vis Sci, 2007, 48(3):1164–1172.
[8] Smith RS, Zabaleta A, Savinova OV, John SW. The mouse anterior chamber angle and trabecular meshwork develop without cell death. BMC Dev Biol, 2001, 1:3.
[9] Teixeira LBC, Zhao Y, Dubielzig RR, Sorenson CM, Sheibani N. Ultrastructural abnormalities of the trabecular meshwork extracellular matrix in Cyp1b1-deficient mice. Vet Pathol, 2015, 52(2):397–403.
[10] Zhao Y, Wang S, Sorenson CM, Teixeira L, Dubielzig RR, Peters DM, Conway SJ, Jefcoate CR, Sheibani N. Cyp1b1 mediates periostin regulation of trabecular meshwork development by suppression of oxidative stress. Mol Cell Biol, 2013, 33(21):4225–4240.
[11] Libby RT, Smith RS, Savinova OV, Zabaleta A, Martin JE, Gonzalez FJ, John SWM. Modification of ocular defects in mouse developmental glaucoma models by tyrosinase. Science, 2003, 299(5612):1578–1581.
[12] Vincent AL, Billingsley G, Buys Y, Levin AV, Priston M, Trope G, Williams-Lyn D, Héon E. Digenic inheritance of early-onset glaucoma: CYP1B1, a potential modifier gene. Am J Hum Genet, 2002, 70(2):448–460.
[13] Choudhary D, Jansson I, Schenkman JB. CYP1B1, a developmental gene with a potential role in glaucoma therapy. Xenobiotica, 2009, 39(8):606–615.
[14] Chavarria-Soley G, Sticht H, Aklillu E, Ingelman-Sundberg M, Pasutto F, Reis A, Rautenstrauss B. Mutations in CYP1B1 cause primary congenital glaucoma by reduction of either activity or abundance of the enzyme. Hum Mutat, 2008, 29(9):1147–1153.
[15] Fuse N, Miyazawa A, Takahashi K, Noro M, Nakazawa T, Nishida K. Mutation spectrum of the CYP1B1 gene for congenital glaucoma in the Japanese population. Jpn J Ophthalmol, 2010, 54(1):1–6.
[16] Wurm A, Sock E, Fuchshofer R, Wegner M, Tamm ER. Anterior segment dysgenesis in the eyes of mice deficient for the high-mobility-group transcription factor Sox11. Exp Eye Res, 2008, 86(6):895–907.
[17] Reis LM, Semina EV. Genetics of anterior segment dysgenesis disorders. Curr Opin Ophthalmol, 2011, 22(5):314–324.
[18] Genčík A, Genčíková A, Gerinec A. Genetic heterogeneity of congenital glaucoma. Clin Genet, 1980, 17(4):241–248.
[19] Sarfarazi M, Akarsu AN, Hossain A, Turacli ME, Aktan SG, Barsoum-Homsy M, Chevrette L, Sayli BS. Assignment of a locus (GLC3A) for primary congenital glaucoma (Buphthalmos) to 2p21 and evidence for genetic heterogeneity. Genomics, 1995, 30(2):171–177.
[20] Moore DB, Tomkins O, Ben-Zion I. A review of primary congenital glaucoma in the developing world. Surv Ophthalmol, 2013, 58(3):278–285.
[21] Li N, Zhou Y, Du L, Wei M, Chen X. Overview of cytochrome P450 1B1 gene mutations in patients with primary congenital glaucoma. Exp Eye Res, 2011, 93(5):572–579.
[22] Cardoso MS, Anjos R, Vieira L, Ferreira C, Xavier A, Brito C. CYP1B1 gene analysis and phenotypic correlation in Portuguese children with primary congenital glaucoma. Eur J Ophthalmol, 2015, 25(6):474–477.
[23] López-Garrido MP, Medina-Trillo C, Morales-Fernandez L, Garcia-Feijoo J, Martínez-de-la-Casa JM, García-Antón M, Escribano J. Null CYP1B1 genotypes in primary congenital and nondominant juvenile glaucoma. Ophthalmology, 2013, 120(4):716–723.
[24] Sharafieh R, Child AH, Khaw PT, Fleck B. LTBP2 gene analysis in the GLC3C-linked family and 94 CYP1B1-negative cases with primary congenital glaucoma. Ophthalmic Genet, 2013, 34(1–2):14–20.
[25] Kaur K, Mandal AK, Chakrabarti S. Primary congenital glaucoma and the involvement of CYP1B1. Middle East Afr J Ophthalmol, 2011, 18(1):7–16.
[26] Waardenburg PJ. [Familial occurrence and hereditary aspects of presenile & senile glaucoma]. Genetica, 1950, 25(1–2): 79–125.
[27] Briard ML, Feingold J, Kaplan J, Bonaiti C, Aron JJ, Blanck MF, Frezal J. Génétique du glaucome congenital: une étude portant sur 231 cas [The genetics of congenital glaucoma: a study of 231 cases]. J Genet Hum, 1976, 24(Suppl):107–123.
[28] Demenais F, Elston RC, Bonaiti C, Briard ML, Kaplan EB, Namboodiri KK. Segregation analysis of congenital glaucoma: approach by two differential models. Am J Hum Genet, 1981, 33(2):300–306.
[29] Morton NE. Heterogeneity in nonsyndromal congenital glaucoma. Am J Med Genet, 1982, 12(1):97–102.
[30] Akarsu AN, Turacli ME, Aktan SG, Hossain A, Barsoum-Homsy M, Chevrette L, Sayli BS, Sarfarazi M. Exclusion of primary congenital glaucoma (Buphthalmos) from two candidate regions of chromosome arm 6p and chromosome 11. Am J Med Genet, 1996, 61(3):290–292.
[31] Akarsu AN, Turacli ME, Aktan SG, Barsoum-Homsy M, Chevrette L, Sayli BS, Sarfarazi M. A second locus (GLC3B) for primary congenital glaucoma (Buphthalmos) maps to the 1p36 region. Hum Mol Genet, 1996, 5(8):1199–1203.
[32] Stoilov I, Akarsu AN, Alozie I, Child A, Barsoum-Homsy M, Turacli ME, Or M, Lewis RA, Ozdemir N, Brice G, Aktan SG, Chevrette L, Coca-Prados M, Sarfarazi M. Sequence analysis and homology modeling suggest that primary congenital glaucoma on 2p21 results from mutations disrupting either the hinge region or the conserved core structures of cytochrome P4501B1. Am J Hum Genet, 1998, 62(3):573–584.
[33] Stoilov I, Akarsu AN, Sarfarazi M. Identification of three different truncating mutations in cytochrome P4501B1 (CYP1B1) as the principal cause of primary congenital glaucoma (Buphthalmos) in families linked to the GLC3A locus on chromosome 2p21. Hum Mol Genet, 1997, 6(4): 641–647.
[34] Stoilov IR, Sarfarazi M. The third genetic locus (GLC3C) for primary congenital glaucoma (PCG) maps to chromosome 14q24.3. ARVO Annual Meeting Abstract, Invest Ophthalmol Vis Sci, 2002, 43(13):3015.
[35] Ali M, McKibbin M, Booth A, Parry DA, Jain P, Riazuddin SA, Hejtmancik JF, Khan SN, Firasat S, Shires M, Gilmour DF, Towns K, Murphy AL, Azmanov D, Tournev I, Cherninkova S, Jafri H, Raashid Y, Toomes C, Craig J, Mackey DA, Kalaydjieva L, Riazuddin S, Inglehearn CF. Null mutations in LTBP2 cause primary congenital glaucoma. Am J Hum Genet, 2009, 84(5):664–671.
[36] Kelberman D, Islam L, Jacques TS, Russell-Eggitt I, Bitner-Glindzicz M, Khaw PT, Nischal KK, Sowden JC. CYP1B1-related anterior segment developmental anomalies novel mutations for infantile glaucoma and von Hippel's ulcer revisited. Ophthalmology, 2011, 118(9):1865–1873.
[37] Vincent A, Billingsley G, Priston M, Williams-Lyn D, Sutherland J, Glaser T, Oliver E, Walter MA, Heathcote G, Levin A, Héon E. Phenotypic heterogeneity of CYP1B1: mutations in a patient with Peters' anomaly. J Med Genet, 2001, 38(5):324–326.
[38] Colomb E, Kaplan J, Garchon HJ. Novel cytochrome P450 1B1 (CYP1B1) mutations in patients with primary congenital glaucoma in France. Hum Mutat, 2003, 22(6):496.

[39] Badeeb OM, Micheal S, Koenekoop RK, den Hollander AI, Hedrawi MT. CYP1B1 mutations in patients with primary congenital glaucoma from Saudi Arabia. BMC Med Genet, 2014, 15:109.
[40] Désir J, Sznajer Y, Depasse F, Roulez F, Schrooyen M, Meire F, Abramowicz M. LTBP2 null mutations in an autosomal recessive ocular syndrome with megalocornea, spherophakia, and secondary glaucoma. Eur J Hum Genet, 2010, 18(7):761–767.
[41] Narooie-Nejad M, Paylakhi SH, Shojaee S, Fazlali Z, Rezaei Kanavi M, Nilforushan N, Yazdani S, Babrzadeh F, Suri F, Ronaghi M, Elahi E, Paisán-Ruiz C. Loss of function mutations in the gene encoding latent transforming growth factor beta binding protein 2, LTBP2, cause primary congenital glaucoma. Hum Mol Genet, 2009, 18(20):3969–3977.
[42] Mohanty K, Tanwar M, Dada R, Dada T. Screening of the LTBP2 gene in a north Indian population with primary congenital glaucoma. Mol Vis, 2013, 19:78–84.
[43] Lim SH, Tran-Viet KN, Yanovitch TL, Freedman SF, Klemm T, Call W, Powell C, Ravichandran A, Metlapally R, Nading EB, Rozen S, Young TL. CYP1B1, MYOC, and LTBP2 mutations in primary congenital glaucoma patients in the United States. Am J Ophthalmol, 2012, 155(3):508–517.
[44] Muskhelishvili L, Thompson PA, Kusewitt DF, Wang C, Kadlubar FF. *In situ* hybridization and immunohistochemical analysis of cytochrome P450 1B1 expression in human normal tissues. J Histochem Cytochem, 2001, 49(2):229–236.
[45] Dong H, Shertzer HG, Genter MB, Gonzalez FJ, Vasiliou V, Jefcoate C, Nebert DW. Mitochondrial targeting of mouse NQO1 and CYP1B1 proteins. Biochem Biophys Res Commun, 2013, 435(4):727–732.
[46] Economopoulos KP, Sergentanis TN. Three polymorphisms in cytochrome P450 1B1 (CYP1B1) gene and breast cancer risk: a meta-analysis. Breast Cancer Res Treat, 2010, 122(2): 545–551.
[47] Mahadevan B, Luch A, Atkin J, Haynes M, Nguyen T, Baird WM. Inhibition of human cytochrome P450 1B1 further clarifies its role in the activation of dibenzo[*a*,*l*]pyrene in cells in culture. J Biochem Mol Toxicol, 2007, 21(3):101–109.
[48] Choudhary D, Jansson I, Sarfarazi M, Schenkman JB. Physiological significance and expression of P450s in the developing eye. Drug Metab Rev, 2006, 38(1–2):337–352.
[49] Stoilov I, Rezaie T, Jansson I, Schenkman JB, Sarfarazi M. Expression of cytochrome P4501b1 (Cyp1b1) during early murine development. Mol Vis, 2004, 10:629–636.
[50] Cvekl A, Wang WL. Retinoic acid signaling in mammalian eye development. Exp Eye Res, 2009, 89(3):280–291.
[51] Brockes JP. Retinoids, homeobox genes, and limb morphogenesis. Neuron, 1989, 2(4):1285–1294.
[52] Caldwell MD, Hu SSJ, Viswanathan S, Bradshaw H, Kelly MEM, Straiker A. A GPR18-based signalling system regulates IOP in murine eye. Br J Pharmacol, 2013, 169(4):834–843.
[53] Saccà SC, Pulliero A, Izzotti A. The dysfunction of the trabecular meshwork during glaucoma course. J Cell Physiol, 2015, 230(3):510–525.
[54] Tang Y, Scheef EA, Wang S, Sorenson CM, Marcus CB, Jefcoate CR, Sheibani N. CYP1B1 expression promotes the proangiogenic phenotype of endothelium through decreased intracellular oxidative stress and thrombospondin-2 expression. Blood, 2009, 113(3):744–754.
[55] Conway DE, Sakurai Y, Weiss D, Vega JD, Taylor WR, Jo H, Eskin SG, Marcus CB, McIntire LV. Expression of CYP1A1 and CYP1B1 in human endothelial cells: regulation by fluid shear stress. Cardiovasc Res, 2009, 81(4):669–677.
[56] Weng J, Luo J, Cheng X, Jin C, Zhou X, Qu J, Tu L, Ai D, Li D, Wang J, Martin JF, Amendt BA, Liu M. Deletion of G protein-coupled receptor 48 leads to ocular anterior segment dysgenesis (ASD) through down-regulation of Pitx2. Proc Natl Acad Sci U S A, 2008, 105(16):6081–6086.
[57] Yin HC, Tseng HP, Chung HY, Ko CY, Tzou WS, Buhler DR, Hu CH. Influence of TCDD on zebrafish CYP1B1 transcription during development. Toxicol Sci, 2008, 103(1):158–168.
[58] Perry KJ, Johnson VR, Malloch EL, Fukui L, Wever J, Thomas AG, Hamilton PW, Henry JJ. The G-protein-coupled receptor, GPR84, is important for eye development in *Xenopus laevis*. Dev Dyn, 2010, 239(11):3024–3037.
[59] Matteson PG, Desai J, Korstanje R, Lazar G, Borsuk TE, Rollins J, Kadambi S, Joseph J, Rahman T, Wink J, Benayed R, Paigen B, Millonig JH. The orphan G protein-coupled receptor, Gpr161, encodes the vacuolated lens locus and controls neurulation and lens development. Proc Natl Acad Sci U S A, 2008, 105(6):2088–2093.
[60] Davenport AP, Alexander S, Sharman JL, Pawson AJ, Benson HE, Monaghan AE, Liew WC, Mpamhanga C, Battey J, Benya RV, Jensen RT, Karnik S, Kostenis E, Spindel E, Storjohann L, Tirupula K, Bonner TI, Neubig R, Pin JP, Spedding M, Harmar A. Class A orphans. International Union of Basic and Clinical Pharmacology/British Pharmacological Society (IUPHAR/BPS) Guide to Pharmacology, 2016, available at http://www.guidetopharmacology.org/GRAC/FamilyDisplayForward?familyId=16.
[61] Wang A, Savas U, Stout CD, Johnson EF. Structural characterization of the complex between alpha-naphthoflavone and human cytochrome P450 1B1. J Biol Chem, 2011, 286(7): 5736–5743.
[62] Choudhary D, Jansson I, Rezaul K, Han DKM, Sarfarazi M, Schenkman JB. Cyp1b1 protein in the mouse eye during development : an immunohistochemical study. Drug Metab Dispos, 2007, 35(6):987–994.
[63] Wallace DM, Pokrovskaya O, O'Brien CJ. The function of matricellular proteins in the lamina cribrosa and trabecular meshwork in glaucoma. J Ocul Pharmacol Ther, 2015, 31(7): 386–395.
[64] Uva MG, Avitabile T, Reibaldi M, Bucolo C, Drago F, Quaranta L, Lionetti E, Longo A. Long-term efficacy of latanoprost in primary congenital glaucoma. Eye (Lond), 2014, 28(1):53–57.
[65] Bejjani BA, Xu L, Armstrong D, Lupski JR, Reneker LW. Expression patterns of cytochrome P4501B1 (Cyp1b1) in FVB/N mouse eyes. Exp Eye Res, 2002, 75(3):249–257.
[66] Doshi M, Marcus C, Bejjani BA, Edward DP. Immunolocalization of CYP1B1 in normal, human, fetal and adult eyes. Exp Eye Res, 2006, 82(1):24–32.
[67] Zhao Y, Sorenson CM, Sheibani N. Cytochrome P450 1B1 and primary congenital glaucoma. J Ophthalmic Vis Res, 2015, 10(1):60–67.
[68] Lander AD. Morpheus unbound: reimagining the morphogen gradient. Cell, 2007, 128(2):245–256.
[69] Turing AM. The chemical basis of morphogenesis. Philos Trans R Soc Lond B Biol Sci, 1952, 273(641):37–72.
[70] Wolpert L. One hundred years of positional information. Trends Genet, 1996, 12(9):359–364.
[71] Crick F. Diffusion in embryogenesis. Nature, 1970, 225(5231): 420–422.
[72] Cunningham TJ, Duester G. Mechanisms of retinoic acid signalling and its roles in organ and limb development. Nat Rev Mol Cell Biol, 2015, 16(2):110–123.
[73] Chambers D, Wilson L, Maden M, Lumsden A. RALDH-independent generation of retinoic acid during vertebrate embryogenesis by CYP1B1. Development, 2007, 134(7): 1369–1383.
[74] Williams AL, Bohnsack BL. Neural crest derivatives in ocular development: discerning the eye of the storm. Birth Defects Res C Embryo Today, 2015, 105(2):87–95.
[75] Sakai Y, Luo T, McCaffery P, Hamada H, Dräger UC. CYP26A1 and CYP26C1 cooperate in degrading retinoic acid within the equatorial retina during later eye development. Dev Biol, 2004, 276(1):143–157.
[76] Edward D, Al Rajhi A, Lewis RA, Curry S, Wang Z, Bejjani B. Molecular basis of Peters anomaly in Saudi Arabia. Ophthalmic Genet, 2004, 25(4):257–270.
[77] Kumar S, Duester G. Retinoic acid signaling in perioptic mesenchyme represses Wnt signaling *via* induction of Pitx2 and Dkk2. Dev Biol, 2010, 340(1):67–74.
[78] Akiyama T, Gibson MC. Morphogen transport: theoretical and experimental controversies. WIREs Dev Biol, 2015, 4(2):99–112.
[79] Bouhenni RA, Al Shahwan S, Morales J, Wakim BT, Chomyk AM, Alkuraya FS, Edward DP. Identification of differentially expressed proteins in the aqueous humor of primary congenital glaucoma. Exp Eye Res, 2011, 92(1):67–75.
[80] Shimozono S, Iimura T, Kitaguchi T, Higashijima SI, Miyawaki A. Visualization of an endogenous retinoic acid gradient across embryonic development. Nature, 2013, 496(7445):363–366.

[81] Borrás T. Mechanosensitive genes in the trabecular meshwork at homeostasis: elevated intraocular pressure and stretch. In: Tombran-Tink J, Barnstable CJ, Shields MB (eds). Mechanisms of the glaucomas: disease processes and therapeutic modalities. Series "Ophthalmology Research", Humana Press, Totowa, NJ, 2008, 329–362.
[82] Grierson I, Howes RC. Age-related depletion of the cell population in the human trabecular meshwork. Eye (Lond), 1987, 1(Pt 2):204–210.
[83] Lütjen-Drecoll E. Functional morphology of the trabecular meshwork in primate eyes. Prog Retin Eye Res, 1999, 18(1): 91–119.
[84] Bidinost C, Hernandez N, Edward DP, Al-Rajhi A, Lewis RA, Lupski JR, Stockton DW, Bejjani BA. Of mice and men: tyrosinase modification of congenital glaucoma in mice but not in humans. Invest Ophthalmol Vis Sci, 2006, 47(4):1486–1490.
[85] Messina A, Siniscalco A, Puccinelli E, Gervasi PG, Longo V. Cloning and tissues expression of the pig CYP1B1 and CYP2J34. Res Vet Sci, 2012, 92(3):438–443.
[86] Sutter TR, Tang YM, Hayes CL, Wo YY, Jabs EW, Li X, Yin H, Cody CW, Greenlee WF. Complete cDNA sequence of a human dioxin-inducible mRNA identifies a new gene subfamily of cytochrome P450 that maps to chromosome 2. J Biol Chem, 1994, 269(18):13092–13099.
[87] Tsuchiya Y, Nakajima M, Yokoi T. Critical enhancer region to which AhR/ARNT and Sp1 bind in the human CYP1B1 gene. J Biochem, 2003, 133(5):583–592.
[88] Tsuchiya Y, Nakajima M, Kyo S, Kanaya T, Inoue M, Yokoi T. Human CYP1B1 is regulated by estradiol *via* estrogen receptor. Cancer Res, 2004, 64(9):3119–3125.
[89] Boschetti E, Righetti PG. Low-abundance proteome discovery. 1st edition, State of the Art and Protocols, Elsevier, 2013.

Corresponding author
Kyrillus Samaan Shohdy, MB, BCh, Faculty of Medicine, Cairo University, Cairo University Hospitals, Al-Saray Street, El-Maniel, 11451 Cairo, Egypt; Phone 02 01229961016, e-mail: kerosam501@gmail.com

Received: December 15, 2016

Accepted: July 17, 2017