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REVIEW

Updates on Genes and Genetic Mechanisms Implicated in Primary Angle-Closure Glaucoma

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Abstract: Primary angle-closure glaucoma (PACG) is estimated to affect over 30 million people worldwide by 2040 and is highly prevalent in the Asian population. PACG is more severe and carries three times the higher risk of blindness than primary open-angle glaucoma, thus representing a significant public health concern. High heritability and ethnic-specific predisposition to PACG suggest the involvement of genetic factors in disease development. In the recent past, genetic studies have led to the successful identification of several genes and loci associated with PACG across different ethnicities. The precise cellular and molecular roles of these multiple loci in the development and progression of PACG remains to be elucidated. Nonetheless, these studies have significantly increased our understanding of the emerging cellular processes and biological pathways that might provide more significant insights into the disease's genetic etiology and may be valuable for future clinical applications. This review aims to summarize and update the current knowledge of PACG genetics analysis research.

Keywords: angle-closure, candidate genes, extracellular matrix, genetics, glaucoma, GWAS, PACG, pathways, polymorphisms, trabecular meshwork

Introduction

Glaucoma is characterized by the degeneration of retinal ganglion cells (RGCs) and progressive damage of the optic nerve axons, most commonly due to elevated intraocular pressure (IOP), leading to irreversible blindness.[1] It is estimated that glaucoma may affect 111.8 million people globally by 2040.[2] The prevalence of glaucoma varies significantly by geographical regions and ethnic groups. Among the different glaucoma types, the worldwide population affected by primary angle-closure glaucoma (PACG) is estimated to exceed 20 million in 2020 and over 30 million by 2040.[2] Based on recent reports, the PACG burden is estimated to be the highest in Asia (0.73%, 95% credible interval 0.18 to 1.96),[3] including middle-east compared to the Caucasians and Africans. It is also more common (60%) among women.[3,4] The likelihood of severe bilateral visual impairment is three times higher in PACG than primary open-angle glaucoma (POAG) and poses a significant public health concern.[5]

PACG is clinically characterized by an iridotrabecular contact (≥ 270°), resulting in aqueous outflow obstruction and elevated IOP due to closure of an existing narrow angle of the anterior chamber associated with glaucomatous optic neuropathy and visual field changes.[6] Currently, there is no widely accepted classification of PACG for both clinical or research purposes. However, on the basis of clinical phenotypes,

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anatomic configurations, etiology of angle-closure and natural history, PACG can be classified as a pupillary block, plateau iris, or peripheral iris crowding (non-pupillary block), and multiple mechanism pattern.[7,8] Pupillary block is considered to be the principal mechanism in the angle-closure pathogenesis. The PACG eye typically exhibits the following ocular biometric findings: a shallow anterior chamber depth (ACD), increased thickness and more anterior position of the lens, hyperopic refractive error, and short axial length (AL).[8] Other anterior segment parameters, such as trabecular to ciliary process distance, iris volume and thickness, anterior chamber area and volume, and lens vault, are also considered significant risk indicators for angle-closure.[8] Due to the natural course of the disease, PACG diagnosis is often made at the advanced phenotypic stages involving chronic visual loss or acute angle-closure.[1] Unraveling the underlying molecular and cellular mechanisms of PACG etiology or PACG phenotypes might help identify at-risk individuals at the early stage of the disease.

Many different factors affect the progression of the anterior chamber angle from narrow to angle-closure (Figure 1). Advanced aging, female gender, Asian ethnicity, anatomical characteristics, family history, and environmental factors to a certain extent are all considered major predisposing risk factors for PACG.[3,4,8–12] The age and sex-adjusted odds of developing angle-closure were 13.6 times higher in affected patients' siblings.[11] Also, the narrow angle risk was seven times higher in first-degree relatives in Singaporean Chinese with overall heritability of PACG of 60%.[10] The reports of the familial tendency towards the disease and racial differences in predisposition to PACG imply an underlying genetic basis for the development of PACG.[4,9]

The initial linkage mapping of nanophthalmos 1 (*NNO1*) gene on chromosome 11p in a large family with traits of autosomal dominant nanophthalmos, hyperopia, and a severe late-stage phenotype of angle-closure provided the first strong evidence for a causal role of genetic components in the pathogenesis of PACG.[13] Since then, several studies have investigated the association of genes and genetic polymorphisms in PACG using the candidate-gene or genome-wide approaches.

With advances in genomic technologies, genome-wide association studies (GWAS) in the recent past have led to the successful identification of several genes and genetic variants associated with PACG in investigations across different ethnicities.[14,15] While the precise role of these genes and genetic variants in the progression and/or development of PACG is still not completely understood, these studies have opened new perspectives in understanding the emerging cellular processes and biological pathways that

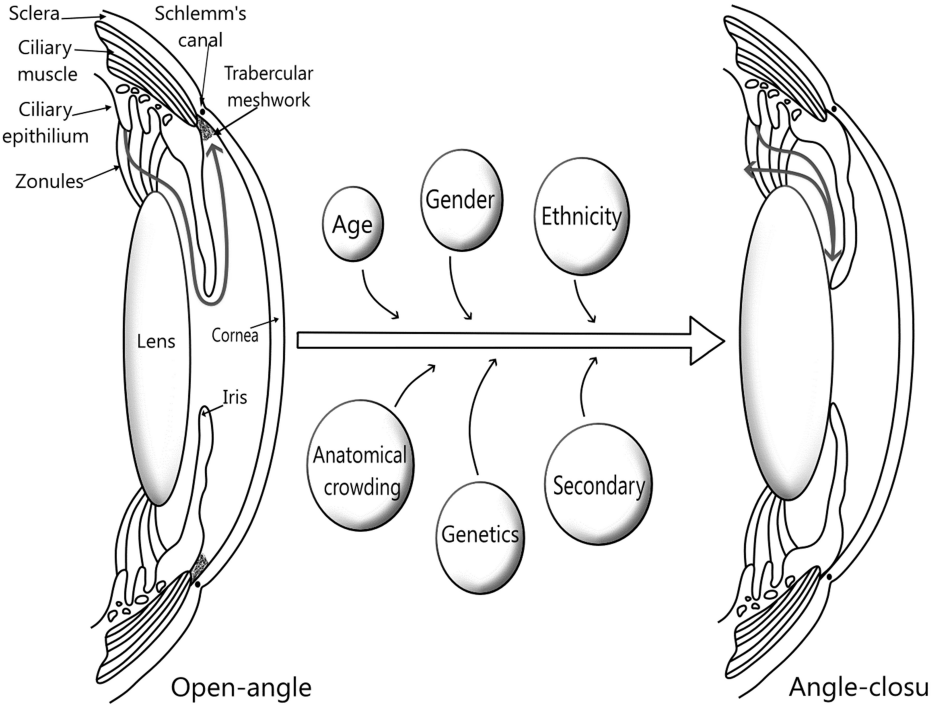

Figure 1 Schematic representation of risk factors contributing to angle-closure in PACG. The curved arrow from the ciliary epithelium indicate the normal flow of aqueous humor in open-angle which is blocked in angle-closure.

might provide greater insight into the genetic etiology of the disease and are the focus of this review with an aim to provide an update on PACG genetic analysis research.

Genome-Wide Association Studies in PACG

GWAS method has been successfully applied to identify genetic loci for POAG[16,17] and exfoliation glaucoma[18] in the past. In regards to the genetics of PACG, two principal GWAS analyses in large populations of multi-ethnicities have lead to the identification of eight susceptibility genetic loci in *PLEKHA7* (pleckstrin homology domain containing A7), *COL11A1* (collagen type XI alpha 1 chain), *PCMTD1* (protein-L-isoaspartate (D-aspartate) O-methyltransferase domain containing 1)-*ST18* (ST18 C2H2C-type zinc finger transcription factor), *EPDR1* (ependymin related 1), *GLIS3* (GLIS family zinc finger 3), *DPM2-FAM102A* (dolichyl-phosphate mannosyltransferase subunit 2, regulatory and family sequence similarity 102 member A, respectively), *CHAT* (choline O-acetyltransferase, also designated as *C10orf53*), *FERMT2* (fermitin family member 2, also known as *PLEKHC1*), all proven to be associated with PACG (Table 1).[19,20]

The first GWAS was conducted by Vithana et al on 1,854 PACG cases and 9,608 control participants from 5 Asian countries (Singapore, Hong Kong, India, Malaysia and Vietnam), with a second-stage replication in additional 1,917 PACG cases and 8,943 controls across six countries, including China, Singapore, India, Saudi Arabia and the United Kingdom.[19] The study reported three novel loci: rs11024102 in *PLEKHA7* (per-allele odds ratio (OR) = 1.22; $P = 5.33 \times 10^{-12}$), rs3753841 in *COL11A1* (per-allele OR = 1.20; $P = 9.22 \times 10^{-10}$) and rs1015213 located between *PCMTD1* and *ST18* locus on chromosome 8q (per-allele OR = 1.50; $P = 3.29 \times 10^{-9}$) to be associated with PACG.[19]

PLEKHA7 on chromosome 11p15 encodes pleckstrin homology domain-containing protein 7, an adherens junction protein that plays a significant role in maintaining the stability of adherens junctions to regulate paracellular permeability and signaling pathways important for biological processes.[21] In the eye, the tight junctions and adherens junctions play a significant role in cell-cell adhesion and paracellular permeability, thus maintaining the stability of structures such as the ciliary body, iris, aqueous flow system, and choroid, which are particularly relevant to glaucoma.[19,22] The specific association of PLEKHA7 with apical junctional complexes (AJCs) and its particular localization to PACG-related anterior segment structures (iris, ciliary body, trabecular meshwork (TM)) and blood-aqueous barrier (BAB) components, including the vascular endothelium within the iris and ciliary microvasculature, indicates that PLEKHA7 might have a potential role for in PACG via fluidic regulation.[23] Changes in the iris volume during pupillary dilation and choroidal effusion have been suggested to be involved in the pathogenesis of PACG.[24] An aberrant fluidic movement in the iris microvasculature and pigmented iris epithelium due to a dynamic increase in iris volume (or lesser reduction in volume) has been observed in angle-closure eyes during dilation.[25] In agreement, Lee et al reported down-regulation of *PLEKHA7* expression in lens and iris of PACG patients, which also correlated with the carriers of the rs11024102 risk allele.[22]

On the other hand, disruption of BAB and leakage of inflammatory proteins and cells into the anterior chamber of the eye was found in both acute and chronic angle-closure glaucoma and suggested contributing to an increase in IOP be another mechanism in angle-closure development.[26] There is evidence to support the role of *PLEKHA7* in BAB maintenance. Silencing of *PLEKHA7* in human non-pigmented epithelial and primary TM cells was shown to affect actin cytoskeleton organization, thereby compromising BAB integrity and aqueous outflow via Rac1/Cdc42 GAP activity of PLEKHA7.[22] Thus, it has been hypothesized that *PLEKHA7* variant may result in reduced expression of PLEKHA7, leading to "leaky" BAB, due to reduced tight junction or adherens junction proteins and altered regulation of fluid dynamics across the Schlemm's canal, resulting in the clinical manifestations of PACG. However, these hypotheses need further validation by in vivo functional studies and animal studies.[19,22,27]

COL11A1 on 1p21.1 encodes one of the two α-chains of type XI collagen, a relatively minor fibrillar collagen. Diseases associated with mutations in this gene include type II Stickler syndrome and Marshall syndrome. These congenital syndromes are associated with manifestations including high myopia and blindness from retinal detachment.[28–30] Collagen contributes to the tissues' structural and mechanical properties, including the TM, sclera, and lamina cribrosa in the optic nerve head.[31] Many studies have highlighted collagen's significant role in high myopia and glaucoma.[32,33] Alterations in the biomechanical features of the extracellular matrix (ECM) due to dysfunctional or structural changes in

Table 1 List of Genes Associated with PACG and Related Phenotypes

Approach / Genes	SNP ID/ Variant(s)	Phenotypes	Chromosomal Location	Possible Role in PACG	References
Linkage					
NNO1	–	Nanophthalmos, hyperopia, ACG	11p13	Ocular development	[13]
Genome-wide					
PLEKHA7	rs11024102	PACG, IOP	11p15.1	Cell adhesion and paracellular permeability, actin cytoskeleton organization	[19,181]
COL11A1	rs3753841 rs1676486 rs12138977	PACG, severity, ACD	1p21.1	Formation of collagen fibrils, ECM organization	[19,86,183]
PCMTD1-	rs1015213	PACG, ACD	8q11.23	Unknown	[19,178,179]
ST18				Proapoptotic, proinflammatory	
FERMT2	rs7494379	PACG	14q22.1	Integrin activation, cell-ECM adhesion, Wnt signaling	[20]
EPDR1	rs3816415	PACG, severity	7p14.1	Cell adhesion, lipid transporter	[20,184]
GLIS3	rs736893	PACG, ACD	9p24.2	Cell survival, Wnt genes activator	[20,183]
DPM2-	rs3739821	PACG	9q34.11	Glycosylation	[20]
FAM102A				Estrogen metabolism, RANK signaling	
CHAT	rs1258267	PACG, ACD	10q11.23	ACh metabolism, autonomic innervations	[15,183]
Candidate genes					
MMP9	rs3918249 rs17576 (rs2664538) rs2250889 rs3918242 (−1562 C>T)	PACG	20q13.12	ECM remodeling	[93,96,97,100,101]
NOS3	Intron 4 VNTR rs7830 rs3918188 rs3793342 rs11771443	PACG, ACD		Oxidative stress, MMP9 activator	[108–111]
HSPA1A (*HSP70*)	rs1043618	PACG	6p21.33	Cell survival, MMP9 activator	[94,108]
HGF	rs1742781 rs5745718	PACG	7q21.11	Emmetropization, Cell survival, c-Met/Wnt signaling	[124,125]

(*Continued*)

Table 1 (Continued).

Approach / Genes	SNP ID/ Variant(s)	Phenotypes	Chromosomal Location	Possible Role in PACG	References
MFRP	rs3814762 rs36015759 rs10790289 rs948414	PACG	11q23.3	Ocular development	[94,132,133]
CHX10	–	PACG	14q24.3	Ocular development	[132]
**TMEM*	–	Dominant nanophthalmos	17q11.2	Ocular development	–
PRSS56	–	Recessive posterior microphthalmia, AL, IOP	2q37.1	Growth and maintenance of ocular drainage tissues and IOP	[204]
CALCRL	rs1157699 Haplotype	Acute PACG	2q32.1	Adrenomedullin regulation	[133,209]
MTHFR	rs1801133 rs1801131	PACG	1p36.22	Remodeling the TM and anterior segment connective tissue	[141]
TNF	rs1800629 rs361525 rs1800630 rs1799724	Primary glaucomas (including PACG)	6p21.33	Apoptosis, proinflammatory	[143]
ABCA1	Haplotypes	PACG	9q31.1	Lipid-mediated repair pathway, neuronal cell death	[145]
MYOC	rs183532 Haplotypes Arg46Stop Pro481Leu Gln368STOP	PACG	1q24.3	Cell-matrix adhesion, misfolding, apoptosis, affect IOP	[149,151,152]
CYP1B1	Leu432Val Haplotype	PACG	2p22.2	Affect IOP, *MYOC* modifier, oxidative homeostasis, TM organization	[149,153]
LTBP2	Gln1417Arg Gly1660Trp	PACG	14q24.3	ECM organization and/or assembly	[154]
SMOC2	rs13208776	PACG	6q27	Regulation of ECM proteins and MMPs	[159]
ACVR1	rs12997	PACG	2q24.1	BMP pathway, Wnt signaling	[161]
Quantitative traits					
ABCC5	rs1401999	PACG, ACD, AL	3q27.1	ACD and AL regulation, ocular development, neuromodulation	[164,166]

(*Continued*)

Table 1 (Continued).

Approach / Genes	SNP ID/ Variant(s)	Phenotypes	Chromosomal Location	Possible Role in PACG	References
RSPO1, C3orf26, LAMA2, GJD2, ZC3H11B, CD55, MIP, ALPPL2, ZNRF3	–	*Refractive error and myopia	1p34.3, 3q12.1 6q22.33, 15q14 22q12.1, 1q32 12q13, 2q37, 1q41	AL regulation	[185]
Animal models					
CFA 8 locus	–	Canine late-onset PACG	8	Unknown	[199]
COL1A2	–	Canine PACG	14	Collagen trimerization	[200]
RAB22A			24	Unknown	
NEB	g.55885214 A->G	Canine PACG	19q	Maintain ciliary muscle tone and iridocorneal angle	[201]
CFA 24 locus	–	Canine PACG	24	Unknown	[202]
CFA 37 locus			37		
PRSS56	–	Mouse ACG-like (microphthalmia AL, IOP)	2q37.1	Growth and maintenance of ocular drainage tissues and IOP	[204]
CALCRL	-	Mouse acute PACG	2q32.1	Adrenomedullin regulation	[206]
NGS (Exome)					
SPATA13	c.1432_1440del; p.478_480del	PACG	13q12.12	Guanine nucleotide exchange factor for GTPase binding proteins, cell adhesion	[196]
COL18A1	c.550G>A, p. Glu184Lys	PACG	21q22.3	ECM organization, Wnt signaling	[186]
Expression studies					
SPARC	–	PACG	5q33.1	Collagen I modulator and ECM remodeling	[160]
COL1A1, VEGFB, VEGFC, VEGFR2	–	PACG	17q21.33, 11q13.1, 4q34.3, 4q12	Fibrosis, angiogenesis	[212]

Note: *Not yet investigated in PACG.
Abbreviations: ACD, anterior chamber depth; Ach, acetylcholine; AL, axial length; BMP, bone morphogenic protein; ECM, extracellular matrix; IOP, intraocular pressure; NGS, next-generation sequencing; TM, trabecular meshwork.

collagen might affect the TM function, resulting in decreased outflow and elevated IOP.[33,34] Variations of collagen levels may lead to inter-individual differences in scleral and lamina cribrosa biomechanical properties.[35,36] These alterations may modify the micro-environment of the optic nerve and possibly increase the susceptibility to axonal injury in glaucomatous eyes.[37,38] Eyes predisposed to PACG are generally small and hyperopic. The *COL11A1* causal variant associated with PACG is hypothesized to alter its function to persuade refractive error development, resulting in smaller hyperopic eyes and thereby predispose to PACG.[19] Besides,

abnormality of collagen in scleral and lamina cribrosa may increase susceptibility to AL changes and predispose to PACG.[39] Considering the presence of *COL11A1* in human TM cells,[40] it is also possible that differential expression of *COL11A1* in the TM may have a critical role in regulating the aqueous outflow pathway to influence the disease risk.[19]

In the *PCMTD1* and *ST18* locus, *PCMTD1* encodes protein-l-isoaspartate O-methyltransferase domain-containing protein 1 of unknown function. The gene *ST18* encodes a protein with zinc finger DNA binding transcription factor activity that functions as a tumor suppressor in breast cancer[41] and a regulator of proapoptotic and proinflammatory genes in fibroblasts.[42] Based on the linkage disequilibrium (LD) analysis and expression levels in the TM, *PCMTD1* was suggested to be a more likely candidate for PACG susceptibility than *ST18*.[19] *PCMTD1-ST18* has been reported to be associated with the primary angle-closure suspect (PACS), providing further support for the role of this locus in angle-closure. PACS is the earliest stage of PACG, suggesting that this locus might increase the risk of narrow angle configurations.[43,44]

The original GWAS investigation[19] was later significantly expanded to over 40,000 participants including 10,503 PACG cases from 24 countries across Asia, Australia, Europe, North America, and South America and is one of the largest GWAS performed so far for any glaucoma types.[20] The study reported evidence of disease association at five new genetic loci upon meta-analysis of all patient collections. These loci included: *FERMT2* rs7494379 (OR = 1.14, $P = 3.43 \times 10^{-11}$) on chromosome 14q22.1, *EPDR1* rs3816415 (OR = 1.24, $P = 5.94 \times 10^{-15}$) on chromosome 7p14.1, *GLIS3* rs736893 (OR = 1.18, $P = 1.43 \times 10^{-14}$) on chromosome 9p24.2, rs3739821 (OR = 1.15, $P = 8.32 \times 10^{-12}$) mapped in between *DPM2–FAM102A* on chromosome 9q34.11, and *CHAT* rs1258267 (OR = 1.22, $P = 2.85 \times 10^{-16}$) on chromosome 10q11.23 (Table 1).

FERMT2 encodes a protein known as pleckstrin homology domain-containing, family C member 1 (PLEKHC1) that belongs to the same pleckstrin family of proteins similar to PLEKHA7, which was associated with PACG in the previous GWAS.[19] It is also known as Kindlin-2 (*KIND2*) and mitogen inducible gene 2 (*MIG2*).[45,46] The protein is a component of the ECM with a role in integrin activation and cell-ECM adhesion.[47] FERMT2 (MIG2), together with migfilin and filamin, has been shown to link cell-matrix adhesions and orchestrate the actin cytoskeleton assembly and cell shape modulation.[48] Besides, FERMT2 (KIND2) has also been demonstrated to interact with ß-catenin and T-cell factor 4 (TCF4) to enhance wingless/integrated (Wnt) signaling.[49] Actin cytoskeleton assembly, cell-to-cell adhesion processes, and Wnt signaling are known to play a significant role in the development and progression of glaucoma, thereby implicating the role of *FERMT2* in PACG pathogenesis.[50–52]

EPDR1 is a lysosomal protein of unknown function. It encodes a glycosylated type II transmembrane protein related to ependymins families of cell adhesion molecules and possibly may have a similar role.[53,54] *EPDR1* has been associated with Dupuytren's disease of the connective tissues.[55] Besides, considering the observed associations between *EDPR1, FERMT2*, and *PLEKHA7* and PACG, the studies suggest a significant role of cell-to-cell adhesion processes in PACG pathogenesis.[19,20] A recent crystal structure study revealed that EPDR1 might function as a lysosomal activator protein or a lipid transporter.[54] Several POAG-linked genetic variants have identified proteins (eg, MYOC, CAV1/2, ABCA1) that play a role in removing or repairing damaged lipids influencing membrane surface tension.[56] EDPR1 may have a similar role in PACG and merits further investigations.

GLIS3 is a transcription factor belonging to the family of Kruppel-like zinc finger proteins. It functions as an activator and repressor of transcription in several cellular processes, including proliferation, apoptosis, differentiation, and development.[57–59] Mutations in *GLIS3* have been associated with diabetes, renal disease, congenital hypothyroidism, and cancers.[60–62] Calderari et al have provided experimental evidence for a pleiotropic role of *GLIS3* in diabetes and neurological disorder and its effect on gene transcription in β-cells and neuron function through regulation of genes involved in autophagy.[63] There is evidence to suggest that the proper functioning of GLIS3 is required for appropriate ß-cell survival.[64] Diabetes is a risk factor for glaucoma, well supported by epidemiological and experimental studies.[65,66] Also, GLIS3 has been demonstrated to function as an upstream transcriptional activator of Wnt signaling genes and induce posterior specification of neural progenitor cells.[59] Although the exact molecular mechanism by which a gene involved in a metabolic pathway may contribute to PACG pathogenesis is unknown, the identification of *GLIS3* variants associated with diabetes and PACG suggests a high relevance of *GLIS3* in cross-phenotype

association and overlapping etiology of complex diseases such as PACG. Besides, it is also possible that GLIS expression might affect the development or cell survival of the anterior segment tissue structures and influence PACG risk.

DPM2 and *FAM102A* are uncharacterized genes mapped between intergenic loci rs3739821on 9q34.11 found to be associated with PACG.[20] *DPM2* encodes a protein involved in glycosylation. It is associated with congenital disorders of glycosylation that cause severe pathological phenotypes related to the nervous system.[67] Altered glycosylation has been reported in glaucomatous TM.[68] These alterations may elicit biological and biochemical changes in the ECM that may contribute to glaucoma pathophysiology. *FAM102A* was identified as an early estrogen-induced gene 1 (also known as *EEIG1*) in response to 17*β*-estradiol, suggesting a role in estrogen metabolism.[69] Accordingly, gender and/or hormonal changes have been observed to influence glaucoma outcomes.[9] Besides, a recent meta-analysis study reported a significant association of the estrogen signaling pathway in open-angle glaucoma with a protective effect of hormone replacement therapy in lowering IOP.[70] Furthermore, EEIG1 (FAM102A) was demonstrated to serve as a novel receptor activator of NF-κB (RANK) signaling component with a role in osteoclast formation,[71] suggesting its role in tissue maintenance, repair, and remodeling. Besides, *PIP5KL1* (Phosphatidylinositol-4-phosphate 5-kinase-like 1) was also found to be the nearby gene on this loci[20] that has been reported to inhibit cell proliferation and migration and may have a role in tumorigenesis.[72]

CHAT encodes an enzyme choline O-acetyltransferase (ChAT), responsible for synthesizing the neurotransmitter acetylcholine (ACh), which has a role in pupillary constriction.[73,74] Studies have demonstrated ChAT presence in amacrine cells but not RGCs.[75,76] However, an alternatively spliced form of ChAT was identified by Tooyama and Kimura[77] and confirmed in rat retina and optic nerve[78] to show that the RGCs also possess a viable ChAT system which may help regulate ACh synthesis and function. Studies suggest the presence of *FOS* (Fos proto-oncogene, AP-1 transcription factor subunit) gene, a transcription factor for Ach that mediates regulation and expression of ChAT and ACh function in the RGCs.[79] ACh functions as a neurotransmitter in both the pre-ganglionic sympathetic and parasympathetic neurons in the autonomic nervous system.[80] Autonomic innervations of the eye control many ocular functions. The autonomic regulation of ocular blood flow, aqueous humor production, and IOP, among others (as reviewed elsewhere[81,82]), can be significant determinants of glaucoma. Interestingly, anticholinergic agents have been found to mediate pupillary block and increase acute PACG risk.[73] Therefore, considering the potential role of the cholinergic system of the eye, it is plausible that natural genetic variation in *CHAT* could alter the risk for PACG by regulating ACh metabolism and influence the autonomic regulation of PACG-related structures.[83]

Numerous replication studies have been performed to validate the GWAS findings[19,20] in other ethnicities with mixed outcomes. Studies have examined these loci for their association with the early-stage angle-closure disease.[43,44,84] Even though *PLEKHA7* (rs11024102), *COL11A1* (rs3753841), and *PCMTD1-ST18* (rs1015213) were strongly associated with PACG among the Asian, these loci were not associated with PAC in a Han Chinese population.[84] A study in the South Indian population consisting of PACS and PAC/PACG patients examined the three variants reported in *PLEKHA7* (rs11024102), *COL11A1* (rs3753841), and *PCMTD1-ST18* (rs1015213) genes.[43] The study failed to replicate the findings of *PLEKHA7* and *COL11A1* but confirmed the earlier reported association between *PCMTD1-ST18* variant and PAC/PACG. Also, none of these 3 variants were found to be associated with PACS. However, in a study by Nongpiur et al in 1397 PACS patients of Chinese ethnicity from Singapore and 604 PACS patients of Indian origin,[44] rs1015213 [A] in *PCMTD1-ST18*, rs3816415 [A] in *EPDR1*, and rs3739821 [G] in *DPM2-FAM102A* showed evidence of significant association in the Chinese cohort. But, only *PCMTD1-ST18* was replicated modestly in the Indian PACS patients. Besides, a meta-analysis showed a significant association of *PCMTD1-ST18* and *DPM2-FAM102A* variants with PACS status,[85] suggesting a possible link of these loci with narrow angle configuration.[44] A study from China consisting of 51 PACG cases and an equal number of controls reported a significant association of *COL11A1* variants, including rs3753841, rs1676486 and rs12138977 with PACG.[86] Also, variant rs3753841 in *COL11A1* was found to be significantly associated with PACG in the Australian cohort ($p=0.017$; OR = 1.34); whereas the *PLEKHA7* variant rs11024102 ($p=0.039$; OR 1.43) and *PCMTD1-ST18* variant rs1015213 ($p=0.014$; OR 2.35) were found to be significantly associated with the disease development in the Nepalese cohort in the same study.[87] However, none of these variants survived multiple testing corrections, indicating a need to investigate large population-based cohorts, nonetheless supporting the

role of these GWAS variants in PACG to a large extent. The meta-analysis studies have shown that *PLEKHA7* rs11024102 is strongly associated with PACG in the Asian population but not among the white population,[85,88] whereas *COL11A1* rs3753841 was significantly associated with PACG both in Caucasian and Asian populations.[85,88] Furthermore, the *PCMTD1-ST18* locus was associated with combined PACS and PAC.[85] In another recent study, the five variants identified by Khor et al[20] were examined in the northeast Iranian PACG patients.[89] Except for the variant rs3739821 in the *DPM2-FAM102A* locus, the study reported a significant association of all other variants in *GLIS3* (rs736893), *EPDR1* (rs3816415), *FERMT2* (rs7494379), and *CHAT* (rs1258267) genes with PACG susceptibility.[89]

Thus far, the GWAS investigations have provided strong evidence for the role of multiple genetic factors in PACG and highlighted the complex and polygenic nature of PACG. Although the findings of the replication studies in other ethnicities are variable, they largely support the role of these genes in PACG.[85,88] The variability in the clinical presentation of PAC/PACG patients, race and sample size are some factors that could contribute to such discrepancies. Besides, the expression of all these genes in the cornea, lens, retina, choroid, and optic nerve tissues lend further support to a significant role of these genes in these structures and plausibly in PACG pathogenesis.[19,20] Nevertheless, the precise pathological function of these genes in PACG is still not completely understood. Future functional studies of these gene products in the ocular system may be able to explain their causal role in the development or progression of PACG.

Candidate Gene Studies in PACG

The candidate gene approach has also been used to identify genes and genetic variants contributing to the pathogenesis of PACG and to replicate the GWAS findings as discussed above. Unlike GWAS, which represents an unbiased genome-wide approach, this method involves investigating genetic associations of one or more allelic variants within a specific target gene or genes hypothesized to have a role in certain traits or phenotype of the disease. Several studies have examined the association between genetic variants in several genes and PACG (Table 1). A meta-analysis by Rong et al revealed polymorphisms in five candidate genes that may contribute to the risk of PACG.[85] These include rs3918249 in matrix metalloproteinases 9 (*MMP9*), rs17427817 and rs5745718 in hepatocyte-growth factor (*HGF*), rs2510143 and rs3814762 in membrane-type frizzled-related protein (*MFRP*), rs7830 in nitric oxide synthase 3 (*NOS3*), and rs1043618 in heat shock protein 70 (*HSP70*).

Considering the significant role of MMPs in ECM turnover in the TM and regulation of aqueous outflow dynamics, MMPs are important candidates in glaucoma and many investigators have examined the association between *MMP9* gene variants and PACG.[90,91] *MMP9* is located on chromosome 20q11.2 and encodes a 92-kDa multidomain enzyme known as gelatinase or type V collagenase, which is actively involved in scleral ECM remodeling.[90,92] Besides the functional evidence in *Mmp9* null mice,[91] molecular genetics analyses across different ethnic groups have reported a variable link between the *MMP9* gene polymorphisms and PACG in Taiwanese,[93] Chinese,[94] Singaporean,[95] Caucasians,[96] Indian[97] and Pakistani[98] populations. Apart from rs3918249 revealed by Rong et al[85] another meta-analysis of six tagged SNPs in *MMP9* by Chen et al showed a significant association between rs17576 (or rs2664538, now merged with rs17576) and non-Chinese PACG patients.[99] The association of this variant was first reported in Taiwanese PACG patients by Wang et al.[93] Variant rs17576 was reported to be in high linkage disequilibrium with rs3918249 (r^2=0.98) and associated with PACG in the Australian and Pakistani cohorts.[96,98] However, the associations were not replicated in Singaporean[95] and Han Chinese[94] ethnicities. However, another study suggested rs3918254 in *MMP9* to be a susceptible locus to PACG in Han Chinese.[100] Likewise, rs2250889 was found to be significant in Southern Chinese PACG patients.[101] Also, a functional promoter variant rs3918242 (−1562C/T), which affected the transcription of the gene and was not a part of the meta-analysis studies, was reported to be associated with PACG patients from North India,[97] but not in PACG patients of Pakistani origin.[98] Variants in *MPP9* may alter MMP9 function and affect ECM restoration during ocular development and may shorten the AL, which is a significant determinant of PACG.[102]

NOS3 is the enzyme responsible for generating nitric oxide, an endogenous signaling molecule, and an emerging therapeutic target for lowering IOP.[103] In the eye, NO synthesis is predominantly localized to the Schelmm's canal cells, and the TM is considered a major site of action.[104] NO is involved in a myriad of physiological processes contributing to vasodilatation, increase local blood flow, and decrease vascular outflow resistance in ocular circulation by activating downstream signaling via soluble guanylate cyclase and cyclic guanosine monophosphate.[105] Also, NO plays a protective role in

oxidative stress-induced tissue injury and apoptosis.[106] However, unlike in POAG, the relationship between vascular dysregulation and PACG is not clearly defined. Furthermore, excessive stimulation of *N*-methyl-d-aspartate (NMDA) receptor (NMDAR) has been shown to induce RGC apoptosis via neuronal nitric oxide synthase (nNOS).[107] This effect of NO on retinal cells was demonstrated to be mediated in part by MMP9 activation through *S*-nitrosylation, corroborated by highly reduced activity of MMP9 in *nNOS*$^{-/-}$ mice. The study suggested a role of NO-activated MMP9 in retinal excitotoxicity.[107] *NOS3* variants have been associated with PACG in the Pakistani (VNTR),[108] Australian (rs3793342, rs3918188, rs7830),[109] but not with PAC/PACG in the Han Chinese population (rs3793342 and rs11771443).[110,111] *NOS3* may have a role in PACG pathogenesis by affecting the anterior chamber depth or activation of the MMP9-related pathway.

HSP70, also known as *HSPA1A* (heat shock protein family A (Hsp70) Member 1A), is an intron-less gene encoding a 70kDa heat shock protein. HSP70 is a stress response protein that functions as a molecular chaperon to regulate protein folding, translocation, misfolding, and degradation to maintain protein homeostasis and cell survival. Hsp70 has been implicated in several neurodegenerative diseases and RGC survival.[112] Variant rs1043618, located at the 5′-untranslated region of *HSPA1A*, downregulates the expression of HSPA1A[113] and has been strongly associated with PACG in the Pakistani[108] and modestly in the Han Chinese[94] populations. Similar to *NOS3, HSPA1A* has also been shown to induce *MMP9* transcription through activation of nuclear factor kappa B (NF-κB) and activating protein-1 (AP-1) and may thus have an indirect role in PACG pathogenesis.[114]

HGF gene located on chromosome 7q21.11 encodes a hepatocyte growth factor protein, which belongs to a family of soluble cytokines and plasminogen-related growth factors. Activated HGF binds to c-Met (mesenchymal-epithelial transition factor) to induce HGF/c-Met signaling and stimulate cell growth, migration, morphogenesis and angiogenesis in numerous cell and tissue types.[115] Over-activation of HGF/c-Met signaling can promote cancer development.[115] HGF/c-Met signaling can stimulate various downstream signaling pathways, including Wnt/β-catenin signaling,[115,116] which is known to have a significant role in glaucoma.[117] In accordance, HGF receptors are expressed in multiple eye tissues, including the TM,[118] and HGF is over-expressed in glaucomatous eyes.[119] HGF has also been shown to regulate the barrier function of retinal pigment epithelium (RPE) cells. Overexpression of HGF in the RPE cells of the rabbit was found to induce retinal detachment.[120] In vitro and in vivo studies have shown that HGF can confer protection to RGCs by increasing neuronal survival and promoting axonal regeneration.[121] HGF was found to confer distinct advantages in sustaining long-term ganglion cell survival and axonal regeneration to respond to favorable stimuli than the two well-established trophic factors, ciliary neurotrophic factor (CNTF) and brain-derived neurotrophic factor (BDNF).[122] Many variants in *HGF* were first reported to be associated with hyperopia and suggested to regulate human ocular development (emmetropization).[123] Since angle-closure glaucoma and hyperopia share the same feature of a short AL, the *HGF* gene has been considered a risk factor of PACG. Studies have shown that variants in *HGF* (rs5745718 and rs17427817) were associated with susceptibility to PACG in the Nepalese[124] and Han Chinese[125] populations. The findings were also corroborated in the meta-analysis by Rong et al.[85] Although the exact role of *HGF* in PACG pathogenesis is unknown, given its role in ocular tissues, it can be speculated that these variants may affect *HGF* expression and over-activate downstream HGF/c-Met/Wnt signaling pathway to induce morphological and physiological changes of the anterior segment structure, disrupting normal aqueous regulation and increase the risk of PACG.

MFRP on chromosome 11q23.3 has been previously associated with microphthalmia, isolated 5,[126] nanophthalmos2[127] and high hyperopia.[127,128] These disorders are characterized by very small, hyperopic eyes exhibiting an unusually short axial length.[129] In humans, MFRP is essential for prenatal ocular growth and postnatal emmetropization, a complicated process involving the regulation of axial growth of the eye in *MFRP*$^{-/-}$ null homozygotes.[130] The strong association of ocular size (small eye) and angle-closure disease makes the genes involved in eye development potential candidates for PACG. Some studies have investigated the association between *MFRP* variants and PACG with inconsistent findings. *MFRP* variants rs3814762, rs36015759, and rs2510143 were not associated with the development of acute angle-closure glaucoma in Taiwanese subjects.[131] No association of *MFRP* was reported by Aung et al in Singaporean Chinese PACG patients.[132] Two variants, rs948414 and rs36015759, and variant rs10790289 in *MFRP* showed nominal association in Australian and Nepalese cohorts, respectively.[133] Likewise, rs3814762 showed modest association in Han Chinese PAC patients.[94]

The meta-analysis by Rong et al confirmed the association of rs3814762 in PACG.[85] MFRP is specifically expressed in the RPE and ciliary body.[134] The gene encodes a glycosylated transmembrane protein with an extracellular frizzled-related cysteine-rich domain and hence speculated to be a regulator of Wnt signaling.[135] Besides, analysis of transgenic mice (*Mfrp*^{rd6}) deficient in Mfrp demonstrated that disruption of Mfrp leads to increased expression of *Prss*56 during postnatal development of the *Mfrp*^{rd6} eye.[136] Given the relation between *Mfrp* deficiency and the expression of *Prss56* (serine protease 56), combined with the genetic link of *MFRP* and *PRSS56* variants and ocular size in humans, the authors suggested a likelihood of these genes being part of a regulatory network that influences postnatal posterior eye maintenance and development.[136] Considering the alleged role of genes involved in ocular development in PACG, Aung et al also explored the role of *VSX2* (visual system homeobox 2; also known as *CHX10*) on chromosome 14q24.3 in PACG patients.[132] Mutations in *VSX2* have been associated with microphthalmia, isolated 2.[137,138] The study identified a possibly disease-causing variant c.728G>A resulting in Gly243Asp substitution in one PACG patient, which was absent in 215 normal controls. However, the overall results did not support a significant role of this variant in PACG.[132] Another gene involved in ocular development that can contribute to PACG and would be worth investigating is *TMEM98* (transmembrane protein 98). Mutations in the *TMEM98* gene have been associated with autosomal dominant nanophthalmos and is expressed in tissues of the outflow pathway.[139,140] So far, this gene has not been investigated in PACG.

Other Candidate Genes in PACG

Besides the genes and genetic variants described above, several other genes/variants have also been investigated in PACG (Table 1). The C677T (rs1801133) and A1298C (rs1801131) polymorphisms of the *MTHFR* (methylenetetrahydrofolate reductase) gene involved in homocysteine metabolism[141] were associated in Pakistani PACG but not in North Indian[142] and Australian and Nepalese cohorts.[133] Promoter variants and haplotypes in *TNF* (tumor necrosis factor), a pluripotent proinflammatory cytokine, were significantly associated with primary glaucoma (including PACG) in the North Indian cohort.[143] Haplotypes in *ABCA1* (ATP binding cassette subfamily A member 1), a gene involved in lipid metabolism and associated with neuroinflammation and neuronal death,[144] were associated in Han Chinese PACG[145] but showed no association in Jordanian Arabs[146] and cohorts of Northern China.[147] *MYOC* (myocilin), *OPTN* (optineurin), *WDR36* (WD repeat domain 36), and *CYP1B1* (cytochrome P450 family 1 subfamily B member 1) genes previously associated with POAG were not associated with the middle-eastern Saudi PACG patients.[148] But a mutation in *MYOC* (Arg46Stop) and *CYP1B1* (Leu432Val) was identified in a Chinese PACG family.[149] In contrast, *MYOC* was found to confer no risk in Chinese PACG cohorts,[150] despite a positive association in the PACG cohort from Quebec.[151] Furthermore, a polymorphism rs183532 and haplotypes in *MYOC* were also associated with PACG in the Han Chinese.[152] On the other hand, *CYP1B1* haplotype (C-C-G-G-T-A) was reported to confer modest risk in PACG patients of Indian origin.[153] Similarly, *LTBP2*, commonly associated with pseudoexfoliation, was observed to have mutations (p.Gln1417Arg and p.Gly1660Trp) that were suggested to contribute to Iranian PACG patients.[154] The studies of genes related to oxidative stress pathways in Saudi PACG patients such as rs4880 in *SOD2* (superoxide dismutase 2),[155] rs1001179 in *CAT* (catalase 2),[156] T1/M0 genotypes in *GST* (glutathione S-transferase),[157] and mitochondrial DNA haplogroups[158] support the role of oxidative stress in PACG. Furthermore, rs13208776 variant in *SMOC2* (secreted protein acidic and rich in cysteine (SPARC)-related modular calcium-binding protein 2) encoding a matricellular glycoprotein and known to regulate the expression of ECM proteins and MMPs has been recently reported to be associated with PACG in the Saudi population.[159,160] Also, rs12997 in *ACVR1* (activin A receptor type I), a critical regulator of the bone morphogenetic protein (BMP)/Wnt signaling, was recently reported to be associated in Saudi PACG patients suggesting a significant role of BMP signaling in PACG.[161]

It is important to note that all of these studies either lack sufficient replication or were reported to have negative associations with PACG and thus need further validations to confirm their role in PACG. Interestingly, none of these candidate genes/variants discussed so far emerged in the GWAS investigations[19,20] conducted in PACG. These discrepancies may primarily result from false-positive signals arising because of the number of samples investigated in these candidate gene studies. Second, it is evident from the GWAS and candidate gene investigation findings that PACG is a genetically complex and multifactorial disease with no clear inheritance pattern. Thus, it is highly likely that variations/genes involved in PACG development and/or progression would be ethnic-specific, as observed in other complex human diseases with no clear Mendelian inheritance. In contrast, the GWAS investigations included

samples consisting of an admixture of different ethnicities (Singaporean Chinese, Hong Kong Chinese, Malaysians, Vietnamese, and Indian) in their discovery stage. As a result, it is possible that population-specific disease association signals could have probably been lost or remains to be verified. More importantly, it is possible that exploring gene-gene interactions between genetic variants to mimic the complex nature of PACG may reveal, at least in parts, the missing heritability in well designed and powered association studies as demonstrated in POAG.[162] Finally, clinical disparities of stages or severity of PACG disease would have confounded the candidate gene and/or GWAS investigation findings.

Quantitative Trait Loci in PACG

Identifying quantitative trait loci (QTL), a genetic link between heritable quantitative traits or endophenotypes in complex multifactorial diseases, is a useful genetic tool to understand the role of a specific trait in the disease pathogenesis and gain further insights into disease mechanisms. Data from genomic studies have identified multiple genetic factors that have the potential to predispose individuals to a high risk of developing PACG. Studies in the past have demonstrated that ocular biometric parameters such as a shallow ACD and short AL are strong anatomical risk factors for PACG.[8,10,163] Associations of the genetic factors with these ocular biometric parameters in PACG have been investigated.

A genome-wide based QTL analysis identified a common genetic variant tagged by marker rs1401999 mapped to *ABCC5* (ATP-binding cassette subfamily C member 5) on chromosome 3 to be associated with ACD (per-allele effect size = −0.045 mm, $P = 8.17\times10^{-9}$) in a case-control dataset from multiple ethnicities across Asia consisting 4276 PACG cases and 18,801 controls.[164] This association was further strengthened when a subgroup of open-angle glaucoma controls was included in the analysis, suggesting that the risk of PACG might, at least, partly be influenced by genetic variants affecting ACD. This variant was also reported to be associated with PACG (OR = 1.13; 95% CI: 1.06–1.22; $P = 0.00046$).[164] The *ABCC5* locus has been further replicated with a moderate association in a group of Chinese PACG patients that investigated the *PARL-ABCC5-HTR3D-HTR3C* region, which is in strong LD at the locus on chromosome 3q27[165] and also recently reported to be nominally associated with AL.[166]

The ABC proteins, a large family of ATPases found in the cell membrane, are involved in the efflux of endogenous metabolites like cyclic nucleotides, folic acid, and other molecules across the cell and participate in tissue defense and cellular signal transduction processes.[167,168] ABCC5 is also known as multidrug resistance protein isoform MRP5, best known for its roles in multidrug resistance observed in chemotherapy-resistant tumors.[169] Studies in *Abcc5*$^{-/-}$ mice showed that ABCC5 is a general glutamate conjugate and analog transporter that affects the disposition of endogenous metabolites, toxins, and drugs.[170] ABCC5 is ubiquitously expressed, including the structures of the anterior segment of the eye such as iris, ciliary body, lens, and cornea.[164,171] The exact mechanism(s) by which *ABCC5* has a role in angle-closure is not known. The inhibition of endogenous ABCC5 activity in zebrafish showed a significant reduction of body length and ocular size,[172] suggestive of a role of ABCC5 in eye growth and development via the regulation of cGMP signaling, which can potentially influence ACD.[173]

A GWA study predicted that *ABCC5* might be the new susceptibility gene for type 2 diabetes in humans[174] through regulation of glucagon-like peptide-1 (GLP-1) secretion as demonstrated in *Abcc5*$^{-/-}$ mice.[175] Studies in the past have reported a link between type 2 diabetes and glaucomatous optic nerve damage.[65,66] Besides, GLP-1 is expressed in the human retina, and GLP-1 receptor activation was found to prevent retinal neurodegeneration by reducing glutamate excitotoxicity and upregulation of pro-survival pathways indicating a plausible neuromodulatory role of *ABCC5* in PACG through GLP-1 regulation.[176,177]

Among the other common variants identified in PACG through GWAS,[19,20] *PLEKHA7* and *COL11A1* variants showed no association with AL or ACD in the European Prospective Investigation of Cancer-Norfolk eye study,[178] four population-based studies that included three from Singapore Eye Study and one Beijing Eye Study,[179] the Jiangsu eye Study in Han Chinese[84] and in another study from Shanghai.[86] However, the latter study did report a significant association of *COL11A1* variants, including rs3753841, rs1676486, and rs12138977 with moderate-to-severe mean deviation-based glaucoma severity.[86] Likewise, studies suggest a role of *PLEKHA7* in modifying the disease risk via the IOP-related pathway.[180,181] In contrast to these variants, the intergenic PACG susceptibility locus between *PCMTD1* and *ST18* (rs1015213) demonstrated consistent association with shallower ACD

but not with AL in the above mentioned European and Asian population-based studies.[178,179] Also, Wei et al reported no association between these three loci and disease severity or progression in Singaporean Chinese patients.[182]

In another study, the association between all the 8 PACG loci identified by GWAS[19,20] and PACG endophenotypes such as AL and ACD were investigated in the Han Chinese population.[183] The study reported nominal association of *COL11A1* (rs3753841), *CHAT* (rs1258267), and *GLIS3* (rs736893) with ACD (p = 0.023, 0.016, 0.01, respectively). However, these associations did not survive false discovery rate correction for multiple testing. Also, the analysis of multiple variants in *MYOC* and *ABCA1* showed no association with ACD and AL.[147] Likewise, variants in *MFRP* and *HGF* have shown no association with ACD and AL phenotypes. However, variant rs7290117 in *ZNRF3* was suggested to be involved in the regulation of AL.[183] In another recent study, Liu et al investigated the association of the eight susceptibility loci identified in PACG with the disease severity based on the visual field mean deviation.[184] The study examined 436 mild-to-moderate PACG and 206 severe PACG patients. Only variant rs3816415 in *EPDR1* was significantly associated with severe PACG (OR, 2.03; 95% CI, 1.49–2.78; $P = 1 \times 10^{-5}$), suggesting that the *EPDR1* variant may predispose individuals to an aggressive form of PACG. The study also reported that PACG patients with a genetic risk score in the highest quartile have more than a 3-fold risk of developing severe PACG.[184] Similarly, *COL11A1* rs1676484 and rs12138977 polymorphisms have also been associated with disease severity in Chinese PACG patients.[86] Shi et al reported that *NOS3* variant rs11771443 was associated with deeper ACD but not with primary angle-closure (PAC), AL and diopter of spherical power in Han Chinese.[110]

In a genomic QTL meta-analysis of ocular AL conducted in 12,531 Europeans and 8,216 Asians of the Consortium for Refractive Error and Myopia cohorts, Cheng et al identified nine loci significantly related to AL (*RSPO1* [R-spondin 1], *C3orf26* [chromosome 3 open reading frame 26], *LAMA2* [laminin subunit alpha 2), *GJD2* [gap junction protein delta 2], *ZC3H11B* 9 [zinc finger CCCH-type containing 11B], *ZNRF3* [zinc and ring finger 3], *CD55* [CD55 molecule (Cromer blood group)], *MIP* [major intrinsic protein of lens fiber], and *ALPPL2* [alkaline phosphatase, placental like 2]).[185] It is interesting to note that although AL is an important clinical determinant of PACG, none of the loci identified by Cheng et al were reported in PACG GWA studies.[19,20] Likewise, *ABCC5* associated with ACD and PACG[164] also did not emerge in these GWAS investigations.[19,20] Taken together, these findings suggest that the contribution of ACD and AL to PACG pathogenesis are more complex events that are still not completely understood, and the involvement of other clinical attributes in PACG pathogenesis remains to be investigated.

Next-Generation Sequencing Studies in PACG

With advances in next-generation sequencing (NGS), a combination of linkage analysis and whole genome/exome sequencing has been utilized to identify causal genes in families with PACG. Using a linkage and whole-exome sequencing approach, Suri et al identified the genetic cause of iridocorneal angle-closure in three unrelated Iranian families with at least ten individuals diagnosed with PACS, PAC, or PACG.[186] A mutation (c.550G>A, p.Glu184Lys) identified in *COL18A1* (collagen type XVIII alpha 1 chain) at 21q22.3 encoding collagen type XVIII was found to be the cause of angle-closure in the pedigree. The inheritance pattern of angle-closure causing mutations in *COL18A1* was autosomal dominant. Other *COL18A1* mutations were also identified in two unrelated PACS families, lending further support for a causative role of *COL18A1* in angle-closure. However, it is important to note that the individuals of these two unrelated PACS families were parents or grandparents of Knobloch syndrome (KS) patients. KS is a rare autosomal recessive disorder with considerable clinical variability and is classically characterized by severe ocular abnormalities, including high myopia, retinal detachment, and occipital encephalocele that often leads to bilateral blindness.[187] *COL18A1* is a significant gene associated with KS type 1.[187–189] Interestingly, glaucoma has also been observed in patients with KS.[190–192]

COL18A1 is expressed in human eye tissues, including the TM, cornea and ciliary body.[186] The potential significance of *COL18A1* in causing PACG emphasizes the importance of collagen and the ECM structure in glaucoma pathophysiology.[33,193] Type XVIII collagen is a component of the ECM proteins that contain multiple triple-helix domains (collagenous domains) interrupted by non-collagenous domains.[194] The N-terminal of the long isoform of the protein consists of a cysteine-rich frizzled

domain that is homologous to the extracellular part of frizzled receptors involved in Wnt signaling. The exact effect of the p.Glu184Lys mutation on *COLA18A1* function is still unknown but may probably interfere with the regulation of the Wnt signaling pathway. Wnt signaling is involved in the regulation of eyeball size, a trait commonly associated with PACG.[195] And interestingly, *MFRP* that has also been implicated in the etiology of PACG, also contains a frizzled-like domain,[135] provides further support to their role in PACG development.

In another recent study using a combination of linkage and whole-genome sequencing, Waseem et al identified a causal genetic variant in *SPATA13* (spermatogenesis associated protein 13) in a seven-generation PACG family of British origin.[196] A single 9bp in-frame deletion variant (c.1432_1440del; p.478_480del) in *SPATA13* on chromosome 13q12.12 was found to segregate in all the affected individuals of the family with variable expression and decreased penetrance. Additional rare variants in *SPATA13* were also observed in unrelated PACS, PAC or PACG cohorts, including the 9bp deletion, supporting the causal evidence of this variant.[196]

SPATA13, also known as ASEF2 (adenomatous polyposis coli-stimulated guanine nucleotide exchange factor 2), acts as guanine nucleotide exchange factor (GEF) for RhoA, Rac1, and Cdc42 GTPases.[197,198] *SPATA13* transcripts encode a 652 amino acid (SP-652) and 1277 amino acid protein (SP-1277) due to alternate N-terminal splicing, which shows nuclear and cytoplasmic localization with partial co-localization.[196] The transcripts show ubiquitous expression and are highly expressed in the iris, cornea, ciliary body, and retina, the tissues most affected by PACG and suggested to regulate tissue homeostasis.[196] In RPE-cells, SP-1277 showed dramatic redistribution during various cell division stages, suggesting a role in mitosis.[196] Isoform SP-652 has been shown to regulate actin cytoskeletal reorganization[198] and is involved in angiogenesis.[198] The 9bp deletion reported in this study was found to increases the Rac1-dependent GEF activity, an effect that was consistent with three other variants reported in this study.[196] Taken together, SPATA13 was suggested to have a regulatory role in cell division and cell adhesion in the anterior segment of the eye, affecting tissue homeostasis and influencing PACG pathogenesis.[196] Similar to SPATA13, PLEKHA7, another protein implicated in PACG,[19,23] encodes a Rac1/Cdc42 GAP activity,[22] implicating the role of the Rho-GTPase pathway in PACG.

Animal-Model Studies in PACG

Further insights into the genetic predisposition towards PACG have also been gained through genetic analysis of animal models. An initial genome-wide study in a Dandie Dinmont Terrier cohort representing a late-onset form of PACG led to identifying a novel 9.5Mb susceptibility locus on canine chromosome 8.[199] Newer susceptibility genes have also been identified as possible contributors to acute PACG in American Basset Hounds. These include: *COL1A2* (collagen type I alpha 2 chain) on chromosome 14, *RAB22A* (RAB22A, member RAS oncogene family) on chromosome 24[200] and *NEB* (nebulin) mapped to chromosome 19q.[201] *NEB* variant (g.55885214 A->G) identified by exome sequencing was also associated with PACG in the second cohort of unrelated Basset Hounds.[201] Given the significant role of collagen in glaucoma[33] and the identification of a collagen gene (*COL11A1*) variant in GWAS[19] implies that *COL1A2* might also represent a highly potential candidate in PACG pathogenesis. RAB22A, are members of the Ras-related family of GTPases and their role in glaucoma is unknown.[200] NEB is a large protein that promotes the contractile function of sarcomeres and is prominently expressed in the ciliary eye muscles, indicating its role in the maintenance of ciliary muscle tone and iridocorneal angle.[201]

In a recent genome-wide and RNA sequencing study in European Basset Hounds, Oliver et al identified two novel loci of 1.4 and 0.2 Mb regions, on chromosomes 24 and 37, respectively, that were significantly associated with PACG and also revealed differential expression of eight genes within these two loci.[202] The locus on chromosome 24, consisting of *RNF24*/*PANK2* (ring finger protein 24 and pantothenate kinase 2, respectively), the nearest upstream genes, has previously been associated with glaucoma traits in humans.[203] However, this study failed to replicate the previous GWAS findings of *NEB* (including the variant), *COL1A2*, and *RAB22A* associations observed in American Basset Hounds,[200,201] probably due to differences in the frequency distribution of the risk loci in the American and European Basset Hounds.[202]

Animal-based studies have also been utilized to investigate genetic links in AL regulation.[204] The genetic alterations in a novel serine protease-encoding gene (*Prss56*) were found to be associated with decreased AL in a mouse model resembling ACG-like phenotype.[204] Besides, mutations in human *PRSS56* in six families with autosomal recessive posterior microphthalmia were also found to

cause a significant reduction in the ocular AL in the same study.[204] *Prss56* is expressed in the retina.[204] Furthermore, *Prss56*$^{-/-}$ mice have been recently shown to exhibit ocular angle defects and increased risk of high IOP.[205] The study suggested a critical role for PRSS56 in the development and maintenance of ocular drainage tissues and IOP homeostasis.[205]

In a transgenic mouse model of acute PAC, overexpression of calcitonin receptor-like receptor (*CALCRL*) in the pupillary sphincter muscle exhibited pupillary palsy due to relaxant effect of adrenomedullin leading to obstruction of aqueous outflow and acutely and transiently elevated IOP, resembling the phenotypic characteristics of acute PACG in humans.[206,207] It was hypothesized that defective adrenomedullin regulation in the pupillary sphincter muscle might result in the development of an acute attack of angle-closure in humans. CALCRL belongs to a family of G-protein-coupled receptors. The transport of CALCRL to the plasma membrane is facilitated by receptor-activity-modifying proteins (RAMPs). Depending on which RAMP members are expressed, CALCRL can function as either a calcitonin-gene-related peptide (CGRP) receptor via RAMP1 or an adrenomedullin receptor via RAMP2.[208] CALCRL and RAMP2 (receptor-activity-modifying protein 2) heterodimers have been identified in the pupillary sphincter muscle.[206] In agreement with the animal studies data, a common variant rs1157699 was nominally associated with acute but not chronic PACG in the Southern Chinese population.[209] However, a rare haplotype AATACAGAT in the *CALCRL* gene was found to exhibit a significant protective effect in the Australian Caucasian cohort (corrected p-value=0.024).[133] The implications of this association are still unclear and were absent in the Nepalese PACG cohort in the same study, indicating a need for replication in other ethnicities and further validation for the role of this gene in human PACG.[133]

Expression Studies in PACG

There are no reports of blood-based or tissue-based mRNA expression profiling studies in PACG. However, few studies have been conducted to investigate altered gene expression of the iris in glaucoma. Among several ocular biometric risk factors associated with PACG, anterior segment optical coherence tomography studies have reported the association between angle-closure and increased quantitative iris parameters such as iris curvature, iris area, and iris thickness.[210,211] These studies suggest biomechanical and structural changes of the iris as significant clinical determinants of PACG. Based on these observations, it was hypothesized that a differential iris response to pupil dilation and choroidal effusion occurrence might have a role in PACG pathogenesis.[212] Increased expression of *SPARC* (secreted protein, acidic and rich in cysteine) and collagen I transcripts were reported in PACG iris than POAG.[160] This finding was in agreement with the previously observed increased deposition of mature collagen I in PACG iris.[213] The study reinforced the established role of SPARC as a modulator of collagen I production and suggested increased levels of iris collagen to be a discrete biological signature for PACG.[160] *SPARC*, a collagen-binding matricellular glycoprotein, is involved in regulating collagen deposition in the ECM and was proposed to play a role in PACG by influencing biomechanical properties of the iris through ECM reorganization.[160,214] In another study, Seet et al examined the expression of known genes implicated in glaucoma, such as *COL1A1* (collagen type 1 alpha 1 chain), *VEGFA, VEGFB, VEGFC* (vascular endothelial growth factors A, B and C, respectively) and members of the VEGF receptors 1 (*VEGFR1*) and 2 (*VEGFR2*).[212] The study reported distinct increased mRNA expression of *COL1A1, VEGFB, VEGFC*, and *VEGFR2* in PACG iris than POAG. Furthermore, a combination of gene expression levels and biometric features such as lens vault and anterior chamber volume augmented cross-validated differentiation between PACG and POAG more effectively with the highest accuracy. The study highlighted distinct molecular disparities between glaucoma types and the importance of combining molecular profiles with known biometric ocular features to gain a better understanding of the disease etiology and their subtypes.[212] A functional and pathway enrichment analysis study of differently expressed genes from two different datasets[215] revealed three important differentially expressed genes, including *HGF, AKR1B10* (aldo-ketoreductase family 1, member B10), and *AKR1C3* (aldo-ketoreductase family 1, member C3) that were suggested to serve as important biomarkers and targets for glaucoma diagnosis and treatment.[216] A more detailed and systematic gene expression studies are needed to identify differentially expressed functional targets in PACG. These transcripts may allow the identification of their regulatory counter-parts such as microRNAs (miRNAs) using in-silico bioinformatics tools. The functional characterization of mRNA-miRNA co-expression patterns can shed light on the pathological mechanisms of the disease, and their

molecular signatures might possibly serve as diagnostic markers.

Potential Pathways Involved in PACG

Genetic studies performed thus far have yielded significant genes contributing to PACG. The proteins encoded by the current set of PACG genes are involved in a broad range of cellular processes and biological functions. Based on these functions, a simple pathway-based enrichment analysis using Enrichr online tool (https://maayanlab.cloud/Enrichr/) predicted overrepresentation of the syndecan-1, ECM organization and integrin-1 pathways to be the top 3 ranking pathways among others that may contribute to PACG pathogenesis (Figure 2).

The ECM of the TM comprises of collagens, elastin-containing microfibrils, matricellular and structural organizing proteins, glycosaminoglycans (GAGs), and proteoglycans that are essential for maintaining the integrity of the TM.[217] ECM is believed to be a critical component to outflow resistance and IOP regulation in normal and glaucomatous eyes.[218] Matrix remodeling constitutes an essential aspect of mechanically regulated pathways.[219] Forces created by high IOP probably induce mechanical stretching or distortion of the ECM that may lead to clinical manifestations of the disease.[219] Although no genetic variants significantly associated with PACG were directly linked to the ECM, several genetic loci were found to be associated with this process, including *COL11A1, COL18A1, SPARC, COL1A2, MMP9, LAMA2* and *LTBP2* as discussed earlier.

Cell adhesion proteins, cell surface ECM receptors, and related binding proteins are also present in the TM beams.[217] Many biological activities of ECM are mediated via integrin-ECM interactions.[220] Integrins are membrane-spanning receptors that mediate cell adhesion to ECM proteins and provide critical connections between actin-mediated cell processes and the ECM (eg, *PLEKHA7, FERMT2, EPDR1*).[221] Syndecans are a family of cell surface ECM receptors expressed in the TM.[222] Syndecan-1 (CD138) is a heparan sulfate proteoglycan that binds to bind to integrins, collagens, associates with actin cytoskeletal structures, growth factors (such as HGF) and participates in diverse biological responses including cell-cell and cell-ECM adhesions, apoptosis, growth factor regulation and angiogenesis.[223] Syndecan-1 has been shown to modulate matrix assembly in several models of inflammatory diseases.[224] Deficiency of *Sdc1*$^{-/-}$ was associated with the assembly of a disorganized matrix and impaired collagen cross-linking in the myocardial infarction mice model.[225] It is tempting to speculate that these proteoglycans may have a similar effect in PACG eyes.[213] More investigations are needed to verify this process.

It is not surprising that ECM remodeling is emerging as a critical mechanism for PACG.[217,218] The ECM is a highly dynamic structure, continually undergoing a remodeling process in response to the microenvironment changes regulating cell behaviors. It is essential to understand the mechanisms of ECM remodeling and its regulation; and how the ECM's biomechanical properties influence cell

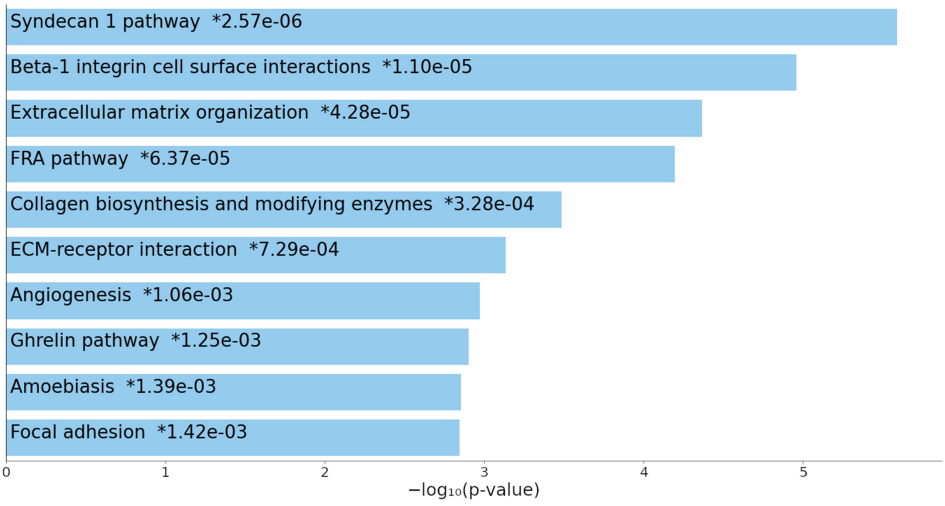

Figure 2 Bar chart showing an overview of pathways significantly overrepresented by a set of genes identified in PACG using Enrichr online tool (https://maayanlab.cloud/Enrichr/). An asterisk (*) next to a p-value indicates the term also has a significant adjusted p-value (<0.05).

behaviors during normal development and in the disease stage. A better understanding of the modulation of ECM dynamics and its components may help develop effective strategies to regulate cell behaviors and maintain tissue integrity and function. These observations also support the role of epistatic gene interactions in PACG.[162]

Concluding Remarks

PACG has long been believed to have a significant genetic premise exhibiting geographical and racial differences in the incidence of PACG. Although the common disease-causing variants/mutations have not been identified yet, the genetics studies have yielded important loci/genes that may contribute towards the pathogenesis of PACG. GWAS investigations have revealed eight genetic loci in *PLEKHA7, COL11A1, PCMTD1-ST18, FERMT2, EPDR1, GLIS3, DPM2-FAM102A*, and *CHAT* that may have a significant role in PACG development or progression. Moreover, additional candidate genes: *HGF, MMP9, MFRP, NOS3*, and *HSPA1A* (*HSP70*) have also been suggested to influence the risk of PACG by meta-analysis. Furthermore, GWAS of quantitative traits has shown that *ABCC5* could be an important marker of PACG phenotype, ACD. Besides, the findings of certain candidate genes (eg, *SMOC2, ACVR1*), animal studies, and expression studies are promising. Genes involved in ECM organization and/or remodeling pathways seem to represent critical targets in PACG pathogenesis. These findings provide a newer perspective in understanding the genetic mechanisms that may plausibly contribute to PAC/PACG and highlight the complex genetic etiology of PACG. They also offer new avenues for future research of proteins encoded by these genes, which participate in a broad range of cellular processes and biological pathways. However, in the absence of any direct functional evidence, the mere presence of these genes in ocular tissues does not confirm their role in PACG etiology. Functional, molecular and animal model studies are further needed to elucidate their causal role in PACG pathogenesis. Also, as is commonly observed in genetic association studies of complex human diseases, most of these susceptibility loci account for a relatively small fraction of PACG cases. They exhibit a moderate effect with an odds of 1.0–1.5 risk per allele, indicating the presence of additional genes/genetic variant-(s) that may have been missed or not covered (eg, insertions, deletions, copy number variants) and the role of gene-gene interactions that remains to be explored. The functions of non-coding RNAs, miRNAs and epigenetic regulators in PACG also remain to be investigated. NGS may represent a useful tool in PACG with strong familial links. The use of integrated genome analysis consisting of whole genome/exome sequencing, transcriptome analysis, and epigenetic regulations might highlight the underlying genetic and environmental abnormalities of clinical relevance. Until then, the importance of accurate clinical phenotyping cannot be overemphasized, and the diagnosis of angle-closure between iris and trabecular meshwork remains the hallmark of PACG.

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Disclosure

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