Contributions of Promoter Variants to Complex Eye Diseases

19

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Abstract

Common eye diseases, including myopia, cataract, glaucoma, and age-related macular degeneration, are the leading cause of blindness and visual impairment, affecting billions of people worldwide. Unlike monogenic diseases, the inheritance of common eye diseases is complex, interplaying with genetics and environmental factors. Genome-wide association studies (GWAS) have identified hundreds of associated genes for common eye diseases; yet, the biological correlation of these disease-associated genes with the pathogenesis of the common eye diseases remains elusive. Apart from the involvement of multiple genes, the epigenetic regulation by environmental factors, including cigarette smoking and sunlight exposure, also determines the occurrence and etiology of the complex diseases. A gene promoter is composed of multiple transcription factor binding sites, which time-dependently regulates the spatial expression of a gene. Genetic variants in the promoter region, creating or disrupting the transcription factor binding sites, could impair the expression of the disease-associated genes and contribute to the pathogenesis of the common eye diseases. In this chapter, the association of the gene variants in the promoter region with the common eye diseases was summarized, with the focus on myopia, cataract, glaucoma, and age-related macular generation. In addition, the contribution of the promoter variants to the pathogenesis of these complex common eye diseases would also be discussed.

Keywords

Promoter · Genetic variants · Myopia Glaucoma · Age-related macular degeneration · Cataract

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19.1 Introduction

Myopia, cataract, glaucoma, and age-related macular degeneration (AMD) are the most common ocular disorders, affecting almost all human beings in the world during their lifetime. No matter what the disease onsets are, the influence of environmental factors, such as sunlight exposure, cigarette smoking and food intake, complicate the development and progression of these common eye diseases [1]. Besides, multiple etiology, clinical heterogeneity, overlap of phenotypic features as well as limited large pedigree also hinder the disease gene discovery for these complex eye diseases. Nevertheless, the major breakthrough in complex eye disease genetics begins with the International HapMap Project and the application of genome-wide association studies (GWAS) on AMD [2]. Since 2005, more than 300 genes were identified as the disease susceptible genes for different eye diseases. Unlike monogenic diseases, disease penetration is hard to be explained by a single associated variant of the disease susceptible gene. Besides, because of the strong linkage disequilibrium, the causal variant cannot be identified only by the statistical methods [3]. Furthermore, considering the late disease onset and interaction with environmental factors, the variants in the exons are less likely to be the causal variants for complex eye diseases.

The precise regulation of gene transcription and translation is the key to the central dogma of molecular biology. This relies on the cis-regulatory DNA elements as well as the epigenetic regulation to control the gene expression. Gene promoters with the enhancers and repressors are composed of multiple transcription factor binding sites, which time-dependently regulate the spatial expression of the genes. Genetic variations in the cis-regulatory elements would create or abolish the transcription factor binding sites, which would influence the transcription of the genes. Cumulative misexpression of the disease susceptible gene could lead to a disease phenotype when age advances. This also explains the small odds ratio (OR) of most GWAS-identified variants for the complex eye diseases. In this chapter, the genetic association of the variants located in the promoter region was summarized, with the focus on the common eye diseases, including myopia, age-related macular generation, glaucoma, and cataract. In addition, the contribution of the promoter variants to the pathogenesis of these complex common eye diseases would also be discussed.

19.2 Myopia

Myopia, one of the most common refractive disorders worldwide, is an epidemic public health issue, especially in Asia. High prevalence (80–90% in young adults; [4]) and fast progression of myopia [5] in East and Southeast Asian countries lead to the national defense and occupational problems as well as the economic burden to the society. Individuals with high myopia, defined as spherical equivalence below –6.0 diopter (D), are more prone to develop serious ocular complications, including macular hole, retinal detachment, glaucoma, premature cataract, and choroidal neovascularization [6], which could lead to irreversible visual impairment or even blindness.

The etiology of myopia is complex. Environmental factors and inheritance have been implicated in the development of myopia. Environmental factors, such as near work, outdoor activities, and sunlight exposure, could be attributed to the development of myopia [7], whereas high heritability of myopia has been observed from the twin and familial studies [8, 9]. Currently, more than 20 *MYP* loci have been mapped for myopia by the family linkage analysis [10]. Moreover, a recent GWAS with 255,925 study subjects identified 161 genetic variants significantly associated with refractive error [11]. These refractive error-associated genes cover the light-dependent signaling cascade from cornea to sclera, including rod-and-cone bipolar synaptic neurotransmission, anterior segment morphology, and angiogenesis. However, the functional consequences of these gene variants to the development of myopia still remain unknown. Besides, most of the associated variants are located in intergenic region, indicating the possible role of transcriptional regulation. In this section, the association of promoter variants in multiple genes with myopia was summarized and discussed.

19.2.1 Paired Box 6 Gene

Paired box 6 (*PAX6*) gene mutations were identified for the development of aniridia [12]; yet, the association of *PAX6* gene with myopia was initially discovered by a genome-wide scan of 506 twin pairs with the heritability of 0.89 in the British population [13]. Significant linkage with a maximum LOD score of 6.1 was identified on chromosome 11p13. Tag SNP analysis demonstrated five variants of *PAX6* gene explaining 0.999 of the haplotype diversity. However, in our Hong Kong Chinese cohort, no sequence alterations in the coding or splicing regions showed an association with high myopia [14]. Besides, tag SNP analysis indicated that there was no significant association of *PAX6* variants (rs2071754, rs3026354, rs3026390, rs628224, rs644242, and rs662702) with mild (–1.0 to –3.0 D), moderate (–3.0 to –6.0 D), and high myopia [15]. These *PAX6* variants were also not correlated with the axial length. In contrast, 2 *PAX6* intron variants (rs2071754 and rs644242) were found to be associated with extreme myopia (< –10.0 D) with odds ratio (OR) of 1.33. Moreover, the *PAX6* rs644242 variant could be associated with high myopia (OR = 0.87; dominant model) as well as extreme myopia (OR = 0.79; dominant model) as suggested by a meta-analysis of 6888 study subjects with Asian ancestry [16].

Although *PAX6* coding variants are not associated with myopia, there could be possibility of genetic variation in the upstream promoter or regulator. Our group identified two highly polymorphic dinucleotide repeats, AC_m and AG_n, in the P1 promoter region of the *PAX6* gene significantly associated with high myopia [14]. Higher numbers of both AC_m and AG_n repeats were observed in high myopia patients with an OR of 1.33. Our luciferase-reporter analysis further demonstrated elevated transcription activity with increasing individual AC_m and AG_n and combined AC_mAG_n repeat lengths, suggesting that higher expression of *PAX6* gene could be related to the development of high myopia.

Apart from the promoter variants, the microRNA binding site could also be involved in the regulation of *PAX6* gene expression. MicroRNA-328 binds to the wild-type C-allele, but not the T-allele of rs644242 variant [17]. Increased microRNA-328 expression suppresses *PAX6* expression and downregulation of *PAX6* reduces scleral cell proliferation. Collectively, promoter and microRNA regulations suggest that increased PAX6 expression is associated with myopia and its pathological changes; therefore, *PAX6* should play a role in myopia development.

19.2.2 Lumican Gene

The correlation of lumican (*LUM*) gene (chromosome 12q21.33) with myopia can be observed from the double knockout mice of lumican and fibromodulin ($Lum^{-/-}/Fmod^{-/-}$), which thinner sclera and increase in axial length were observed in $Lum^{-/-}/Fmod^{-/-}$ mice [18]. Similarly, knockdown of lumican gene (*lum*) in zebrafish by antisense morpholinos resulted in scleral thinning and increased size of scleral coats due to the disruption of the collagen fibril arrangement in the sclera [19]. However, *LUM* is not the candidate gene in the *MYP3* locus for high myopia [20]. On the contrary, a LUM promoter variant rs3759223 was first suggested to be associated with extreme myopia in the Taiwan population with a *p*-value of 2.83×10^{-4} [21]. A meta-analysis with 1545 Chinese subjects from five studies indicated that the C-allele of *LUM* rs3759223 variant is protective against high myopia with an OR of 0.53 [22]. Yet, the *LUM* rs3759223 variant is not associated with high myopia in the Korean population [23]. Another meta-analysis with 2297 subjects from six studies confirmed no association of *LUM* rs3759223 variant with high myopia in all genetic models [24].

In addition to the rs3759223 variant, another *LUM* promoter variant rs3759222 is also not significantly associated with high myopia in the Korean population [23]. In contrast, the haplotypes of *LUM* variants c.601, c.-59, c.-628, and c.-1554 are significantly associated with high myopia in the Taiwan population with an OR of 4.71 [25]. Apart from the promoter variants, a

3′-UTR variant (c.1567:C>T) showed a significant association with high myopia in the Taiwan population [26]. The T-allele of *LUM* c.1567 variant exhibits a lower reporter gene activity compared to the C-allele.

Collectively, although there is controversy in the association of *LUM* promoter variants with myopia, population-specific association could exist for different *LUM* promoter variants.

19.2.3 Extracellular Matrix-Related Genes

Laminin-α1 (*LAMA1*) gene on chromosome 18p11.31 is a candidate gene in the *MYP2* locus for high myopia. However, none of the variants across the *LAMA1* gene, including 2 promoter variants (rs334384 and rs334420), are associated with extreme myopia in the Japanese population [27]. Another *LAMA1* promoter variant rs2089760 has been shown to be associated with high myopia in the Chinese population with an OR of 1.38 [28]. This *LAMA1* promoter variant is located at the transcription factor binding site, which the A-allele of rs2089760 variant, compared to the wild-type G-allele, reduces transcription factor binding ability and transcriptional initiation activity, and negatively regulates the expression of *LAMA1* gene [29]. This indicated that reduced expression by *LAMA1* rs2089760 variant could be involved in the development of pathological myopia.

Although the expression of matrix metalloproteinase-2 (MMP-2), but not MMP-3, was found to be elevated in human aqueous humor of the myopic eyes [30], no significant association was detected for the promoter variants of MMP-1 (c.-1607), MMP-2 (c.-1306:C>T and c.-735C>T), and MMP-3 (c.-1612) with high myopia in the Japanese population [31]. The association of MMPs variants requires further confirmation in different populations.

No association of collagen type I alpha 1 (*COL1A1*) variant was identified with myopia in the Caucasian population [32]. Similarly, there is also no association detected for the *COL1A1* intron variant rs2075555 with high myopia [33]; yet, a meta-analysis of 1620 Asian subjects showed a significant association of *COL1A1* promoter variant rs2269336 with high myopia [34]. Moreover, increased methylation at the 6 cytosine-phosphate-guanine (CpG) sites in the promoter and exon 1 region of *Col1a1* gene was reported in the monocular form deprivation-induced mice, accompanied with reduction of scleral *Col1a1* mRNA when compared to the normal control mice [35]. These indicate that the variation in COL1A1 expression, especially in sclera, could be involved in the development of myopia.

19.2.4 Other Genes

Transforming growth factor-β-induced factor (*TGIF*) was first reported to be associated with high myopia in our Hong Kong Chinese cohort [36]. However, the *TGIF* promoter variant rs4797112 is not associated with ocular biometric measures and myopia in the Australian Caucasian cohort [37].

Myocillin (*MYOC*) is a disease-causing gene for primary open angle glaucoma [38]. Mild association was reported for the *MYOC* variants with high myopia in the Caucasian populations [39]. However, in our Hong Kong Chinese cohort, we did not find the association of a GT repeat from c.-339 to c.-314 in the *MYOC* promoter with myopia [40].

19.3 Age-Related Macular Degeneration

AMD is the leading cause of irreversible blindness and visual impairment in the elderly populations, which will affect 196 million people worldwide in 2020 [41]. According to the international classification and grading system of age-related maculopathy and AMD [42], early AMD is characterized by drusen as well as the hyperpigmentation and hypopigmentation of retinal pigment epithelium (RPE) in the macula. Advanced stage is divided into "non-neovascular" and "neovascular" AMD. Non-neovascular AMD is character-

ized by geographic atrophy of RPE with an oval hypopigmented spot in which large choroidal vessels are visible, whereas neovascular AMD is characterized by choroidal neovascularization (CNV), which could lead to the detachment of the neuroretina or RPE from Bruch's membrane by serous or hemorrhagic fluid. Current effective treatments are limited to the anti-vascular endothelial growth factor (VEGF) treatments against neovascular AMD, and there is still no proven therapy for non-neovascular AMD [43].

AMD is a late-onset and progressive disease. Clinical heterogeneity, overlap of phenotypic features, and gross interactions with environmental factors, such as smoking, body mass index, hypertension, and chronic inflammation, complicate the genetic investigations for AMD [44]. In spite of rare big pedigrees for family linkage analysis, a meta-analysis of genome scans has revealed chromosome 10q26 to be the strongest AMD susceptibility locus, whereas chromosomes 1q, 2p, 3p, and 16 are likely linked to AMD [45]. Yet, the major breakthrough in AMD genetics was achieved by GWAS since 2005. Currently, a large GWAS with 33,976 study subjects from the Caucasian populations identified 52 independently AMD-associated variants across 34 loci [46]. Moreover, the Genetics of AMD in Asians (GAMA) Consortium also identified three additional AMD loci in *C6orf223*, *SLC44A4*, and *FGD6* genes [47]. However, most of the associated variants are located in the intergenic regions or introns, suggesting the possibility of gene expression regulation by the cis-regulatory elements in these loci. In this section, the association of promoter variants in GWAS identified genes with AMD was summarized and discussed.

19.3.1 Complement Factor H Gene

Complement factor H (*CFH*) gene on chromosome 1q31 is the first AMD-associated gene identified by the GWAS analysis [48], which the p.Tyr402His variant (rs1061170) shows the strongest association with AMD in the Caucasian population (OR = 7.4). On the contrary, the p.Ile62Val variant (rs800292), instead of p.Tyr402His, is associated with neovascular AMD in our Hong Kong Chinese population [49]. In addition to the non-synonymous variants, we also identified 2 *CFH* promoter variants rs3753394 (c.-331T>C) and rs35836460 (c.-195T>C) significantly associated with AMD from the whole gene screening analysis [50]. The association of the *CFH* rs3753394 variant with AMD has been confirmed in the Sichuan Chinese [51] as well as the Northern Spanish populations [52]. The haplotype containing the C-allele of *CFH* rs3753394 variant confers a significant protection against AMD. Furthermore, a meta-analysis from 19 studies with 10,676 subjects identified a significant association of another *CFH* promoter variant (rs1410996; c.-543G>A) with AMD [53].

A 241-bp region from c.-416 to c.-175 of *CFH* promoter shows specific transcription factor binding activity with c-Jun and c-Fos in astrocytes [54], implying that *CFH* promoter variants rs3753394 and rs35836460 could influence the transcription and expression of *CFH* gene (Fig. 19.1). This could be further confirmed by another GWAS that *CFH* promoter variant rs3753394is significantly associated with the serum levels of C3 [55], which is negatively regulated by CFH protein. Collectively, *CFH* promoter variants should be involved in the regulation of *CFH* gene expression, which in turn regulates the activation of the alternative complement system by interacting with C3.

19.3.2 High Temperature Requirement Factor A1 Gene

The age-related maculopathy susceptibility protein 2 (*ARMS2*)/high temperature requirement factor A1 (*HTRA1*) locus on chromosome 10q26 is the second AMD-associated locus identified by GWAS from our Hong Kong neovascular AMD cohort [56]. Our previous meta-analysis confirmed the association of *HTRA1* rs11200638 variant (G>A) with AMD globally across different ethnic groups with an OR of 7.32 in the homozygous model [57]. The risk A-allele of *HTRA1* promoter variant rs11200638 variant

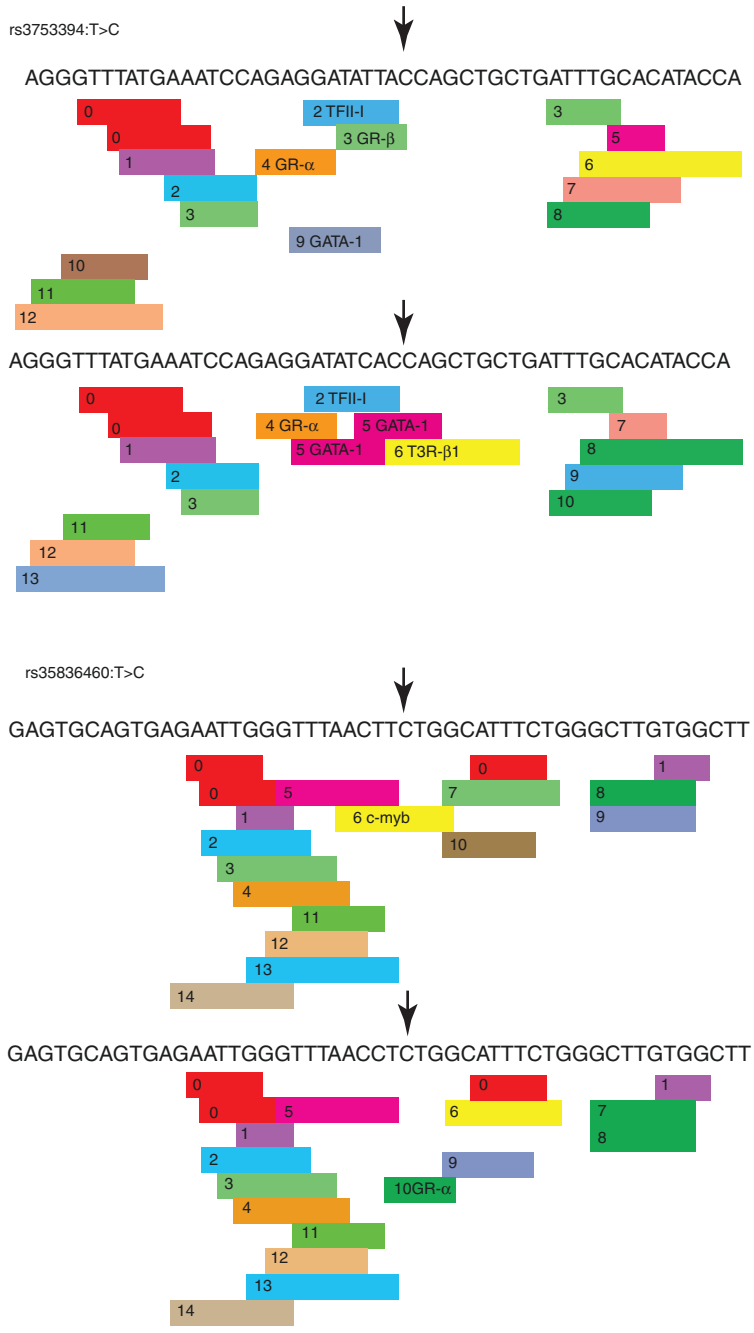

Fig. 19.1 Transcription factor binding site prediction on the AMD-associated complement factor H promoter variants. The GR-β site of the rs3753394 T-allele is predicted to be changed to one GATA-1 site and one T3R-β1 site at the rs3753394 C-allele. The c-Myb site of the rs35836460 T-allele is predicted to be changed to the GR-α site at the rs35836460 C-allele. The transcription factor binding sites were predicted by PROMO (http://alggen.lsi.upc.es/cgi-bin/promo_v3/promo/promoinit.cgi?dirDB=TF_8.3/)

was demonstrated to increase the transcription activity of *HTRA1* promoter [56], and enhanced HTRA1 protein expression was detected in the retina from AMD patients [58]. Moreover, the *HTRA1* promoter variant rs11200638 increases the AMD susceptibility joint addictively with the *CFH* rs800292 variant (OR = 23.3) as well as smoking (OR = 15.71; [59]), but not with the cholesterol level [58]. In addition, the *HTRA1* promoter variant rs11200638 is associated with poorer visual acuity outcomes at 12 months, and the AMD patients with the homozygous AA genotype are more likely to lose more than 15 letters after 12 months [60]. The *HTRA1* promoter vari-

ant rs11200638 is also associated with a poorer response to the ranibizumab and bevacizumab anti-VEGF treatment for neovascular AMD.

Apart from the rs11200638 variant, we identified another common promoter variant rs2672598 (T>C) associated with neovascular AMD by whole gene sequencing analysis in our Hong Kong Chinese cohort [61]. The association of rs2672598 with neovascular AMD is independent of rs11200638; yet, the haplotype of the 2 *HTRA1* promoter variants rs11200638-rs2672598 (AA-CC) confers 43.11-folds of risk to neovascular AMD. Luciferase-report assay demonstrated that the C-allele of *HTRA1* rs2672598 variant shows higher luciferase expression than the wild-type T-allele (Fig. 19.2). In contrast, the luciferase expression levels are similar between the risk A-allele and the wild-type G-allele of *HTRA1* rs11200638 variant. Furthermore, the expression level of HTRA1 protein in vitreous humor with rs2672598 CC genotype was significantly higher than that with the wild-type TT genotype [61], whereas the rs11200638 genotypes are not correlated with the HTRA1 protein expression level in vitreous humor [62]. Furthermore, the C-allele of *HTRA1* rs2672598 variant was predicted to change the transcription factor binding sites of *HTRA1* promoter, whereas the A-allele of rs11200638 variant does not change the transcription factor binding sites. Therefore, we postulate that the *HTRA1* promoter variant rs2672598, instead of rs11200638, should be responsible for the elevated *HTRA1* transcriptional activity and HTRA1 protein expression in the eye.

Besides, an insertion/deletion variant between the *ARMS2* and the *HTRA1* genes significantly induces *HTRA1* transcription regulator activity in photoreceptor cell lines, and the insertion/deletion variant region should be potentially surrounded by transcriptional suppressors and activators [63]. Liquid chromatography-mass spectrometry identified the LYRIC (lysine-rich CEACAM1 co-isolated) protein binding to the insertion/deletion region. In addition, induced pluripotent stem cells from neovascular AMD patients carrying the insertion/deletion variant showed significant upregulation of *HTRA1* transcript compared to the controls. Whether the insertion/deletion variant is in the same risk haplotype with the rs11200638 and rs2672598 variants requires further fine mapping analysis. Collectively, the cis-regulatory variants in the *HTRA1* promoter region likely induce the upregulation of HTRA1 expression. High *HTRA1* expression induces RPE cell death [64], resembling the pathological changes in AMD development.

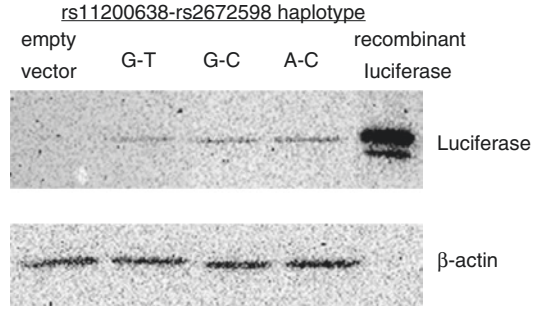

Fig. 19.2 Luciferase expression analysis on the *HTRA1* promoter of the rs11200638-rs2672598 haplotype. Detection of luciferase expression was performed by immunoblotting. The wild-type rs11200638-rs2672598 haplotype (G-T) is the wild type reference. Comparing to the G-T haplotype, elevated luciferase expression was observed for the G-C and A-C haplotypes, indicating that the C-allele of rs2672598 variant enhances the transcription activity of *HTRA1* promoter. In contrast, there was no difference in luciferase expression level between the G-C and the A-C haplotypes, suggesting that the A-allele of rs11200638 would not alter the transcription activity of *HTRA1* promoter. Recombinant firefly luciferase was used as a positive control, whereas empty pGL3 vector was used as a negative control

19.3.3 Tumor Necrosis Factor Receptor Superfamily Member 10A Gene

Tumor necrosis factor receptor superfamily member 10A (*TNFRSF10A*)-*LOC389641* on chromosome 8p21 was first identified as a susceptible locus for neovascular AMD in the Japanese population [65]. The most significantly associated variant (rs13278062: T>G) is located in the promoter region of *TNFRSF10A* gene. In collaboration with the Kyoto Japanese cohort, we validated the association of *TNFRSF10A* promoter variant rs13278062 with neovascular AMD in the Asian

population [66]. However, the association of rs13278062 variant with neovascular AMD was not identified in the Beijing Chinese cohort [67]. A meta-analysis showed a nominal association of *TNFRSF10A* promoter variant rs13278062 with an increased risk of advanced AMD (OR = 1.17). The *TNFRSF10A* promoter variant rs13278062 is also significantly associated with the second-eye involvement in the Japanese population [68]. In addition, the recent large GWAS analysis also identifies the significant association of another *TNFRSF10A* promoter variant rs79037040 with AMD [46], indicating that *TNFRSF10A* expression level variation should be involved in the pathogenesis of AMD.

Although the contribution of gene to the AMD development could be minor (OR = 0.7–0.9), the activator protein 1 binds to the region around rs13278062 and regulates *TNFRSF10A* gene expression [69]. It has been reported that the G-allele of *TNFRSF10A* promoter variant rs13278062 enhances the transcription activity of *TNFRSF10A* promoter when compared to the wild-type T-allele [70]. *TNFRSF10A* gene encodes for TRAIL receptor 1 (TRAILR1), also known as death receptor 4, which is broadly expressed in human RPE and mouse rod photoreceptors [71]. Activation of TRAILR1 can induce apoptosis through caspase-8 pathway [72] as well as the production of inflammatory cytokines and the promotion of inflammation through NF-κB pathway [73]. Dysregulation of *TNFRSF10A* gene expression could be involved in the pathogenesis of AMD.

19.3.4 Lipase C Gene

Lipase gene (*LIPC*, hepatic type) on chromosome 15q21.3 was first identified to be associated with AMD by GWAS analysis in the Caucasian population, which the AMD-associated variants (rs493258 and rs10468017) are located in the promoter region of LIPC gene [74]. The association of *LIPC* promoter variants rs493258 and rs10468017 with advanced AMD is confirmed in two independent Caucasian populations [75]. However, the rs10468017 variant is not associated with advanced AMD in the Indian population [76]. Nevertheless, there could be a possible interaction among *LIPC* rs10468017 variant, *CFH*, and complement factor I (*CFI*) variants in AMD risk prediction [77].

The minor T-allele of *LIPC* rs10468017 variant, with a reduced risk of AMD (OR = 0.4–0.5), reduces the expression of LIPC gene [74], and it is associated with higher levels of serum high-density lipoprotein (HDL; [78]), Although there is a lack of consistent association between HDL alleles and AMD risk, the LIPC and HDL effects could be indirect and accumulative. Changes in HDL-mediated transport of lutein and zeaxanthin could be a possible mechanism by variations in LIPC levels to the risk of AMD [79]. Furthermore, drusen, the hallmark of AMD, also contain cholesterol deposits [80], indicating an aberrant in cholesterol transport. Yet, there are no significant interactions between LIPC and smoking, body mass index (BMI), or lutein [77].

19.3.5 Other Genes

Vascular endothelial growth factor A (*VEGFA*) gene locus on chromosome 6p12 was first confirmed to be associated with advanced AMD in the Caucasian populations by GWAS analysis [81]. Although the *VEGFA* promoter variant rs699947 (A>C) shows no significant association with AMD [82], the C-allele of *VEGFA* rs699947 variant is associated with higher VEGF production [83]. Instead, the C-allele of *VEGFA* rs699947 variant is correlated with better response to ranibizumab treatment than the A-allele in multiple populations [84, 85]. In contrast, the C-allele of *VEGFA* rs699947 variant is significantly higher in photodynamic therapy (PDT) nonresponders than the PDT responders in the Finland population [86].

Interleukin-8 (*IL8*) promoter variant rs4073 (c.-251A/T) was first reported to be associated with AMD in the British population by a candidate gene analysis [87]. This promoter variant is confirmed to be associated with younger onset age of neovascular AMD in the Finland population [88]. Moreover, the *IL8* promoter variant

rs4073 is also associated with persisting fluid in optical coherence tomography [89]. The A-allele of rs4073 variant is more frequent in nonresponders of initial bevacizumab treatment than in responders, and it can predict poorer outcome together with the occult or predominantly classic lesions. The A-allele of *IL8* promoter variant rs4073 is associated with higher levels of circulating and secreted IL-8 protein [90]. Higher IL-8 production could lead to IL-8 stimulated angiogenesis and capillary leakage [91].

Apolipoprotein E (*APOE*) variant (rs2075650) on chromosome 19q13.32 was first suggested to be associated with early AMD by a GWAS meta-analysis [92]. The large GWAS analysis also confirms the significant association of APOE variant (rs429358) with AMD [46]. However, the APOE ε4 genotype is not associated with AMD in our Hong Kong Chinese population [93]. Yet, the extended haplotype analysis demonstrated a significant association of APOE haplotype, including an *APOE* promoter variant rs405509 (G>T), with AMD [94], suggesting that the relative rate of APOE isoform expression would be crucial in AMD pathogenesis based on the influence of *APOE* promoter activity by the rs405509 variant [95]. However, a pooled analysis of 15 studies indicated that the extended haplotype with rs405509 variant does not increase additional risks beyond the ε2 and ε4 haplotypes [96].

Excision repair 6, chromatin remodeling factor (*ERCC6*) promoter variant c.-6530C>G was first reported to be associated with AMD and interact with *CFH* variant rs380390 in the Caucasian population [97]. The putative transcription factor binding site is predicted to be changed in the G-allele of *ERCC6* promoter variant, and the luciferase expression is higher in the G-allele of *ERCC6* promoter variant compared to the wild-type C-allele. Intense ERCC6 expression was also found in AMD eyes with the G-allele of *ERCC6* promoter variant. Another *ERCC6* promoter variant rs3793784 was reported to confer a small increase in risk for advanced AMD in the Dutch populations, but not replicated in two non-European cohorts [98]. In contrast to the c.-6530C>G variant, early AMD-affected donor eyes showed lower ERCC6 expression than healthy donor eyes. Whether increase or decrease in *ERCC6* transcriptional activity contributing to the AMD development requires further investigations.

Serpin family G member 1 (*SERPING1*) variant rs2511989 on chromosome 11q12.1 was first reported to be associated with AMD in the British population by low-density variant screening [99]. The *SERPING1* variant rs2511989 is not associated with AMD in our Hong Kong Chinese population as well as other East Asian populations, but associated with AMD in the Caucasian populations [100]. *SERPING1* gene encodes the C1 inhibitor, which is crucial in inhibiting the complement component 1 (C1) in the classic complement pathway. Although the *SERPING1* promoter variant rs2649663 is not associated with AMD, it is associated with C1 inhibitor levels and higher level of C1 inhibitor was shown in AMD patients compared to the control subjects in the British population [101]. This suggests that *SERPING1* promoter variation could also influence the expression of *SERPING1* gene.

Matrix metalloproteinase-2 (*MMP2*) variant rs2287074 has been shown to be associated with AMD, and. the A-allele is associated with a lower likelihood of AMD in older Caucasian women [102]. An *MMP2* promoter variant rs243865 (c.-1306C>T) was reported to be associated with AMD in the northern Chinese population [103]. However, no association of *MMP2* promoter variant rs243865 with AMD was observed in the Turkish and Lithuania populations [104, 105]. Instead, the *MMP2* promoter variant rs243865 is associated with younger AMD onset in male patients [106]. Besides, the plasma levels of MMP-2 in AMD patients are not significantly different from that of the control subjects [107], indicating that MMP-2 is unlikely play a major role in the pathogenesis of AMD.

Toll-like receptor 3 (*TLR3*) variant rs3775291 on chromosome 4q35.1 was first reported to be associated with non-neovascular AMD in the Caucasian population [108]. However, the *TLR3* promoter variants rs5743303 and rs5743305 are not associated with neovascular AMD in the northern Chinese population [109].

Mice deficient with CC-cytokine ligand 2 (*Ccl2*) gene, also known as monocyte che-

moattractant protein-1, develop the pathological features of AMD, including accumulation of lipofuscin in RPE, the presence of drusen beneath RPE, photoreceptor atrophy as well as CNV [110]. However, the promoter variants c.-2578A>G and c.-2136A>T of CCL2 gene are not associated with AMD in the Netherlands Caucasian population [111].

19.4 Glaucoma

Glaucoma is the leading cause of irreversible blindness and visual impairment, which would affect 76 million people worldwide in 2020 [112]. Primary glaucoma can be subclassified into primary congenital glaucoma, primary open angle glaucoma (POAG), and primary angle closure glaucoma (PACG). They share common pathologies of retinal ganglion cell loss and the axonal degeneration. Although research studies have deciphered most of the glaucoma pathogenesis, elevated intraocular pressure (IOP; >21 mmHg) is the only recognized modifiable risk factor in glaucoma treatment, which the progression of glaucoma can be attenuated when the IOP is lowered by 30–50% [113]. Yet, normal intraocular pressure can also be found in a number of POAG patients [114]. Nevertheless, the IOP lowering treatment is the only proven treatment for all forms of glaucoma [115].

The inheritance of glaucoma has been suggested for 70 years [116]. Earlier studies relied on family linkage analysis to map the disease genes/loci for glaucoma in large pedigrees [117, 118]. Similar to AMD, the discovery of glaucoma-associated genes has been boosted with the application of GWAS. The first GWAS-identified glaucoma gene is the lysyl oxidase-like 1 (*LOXL1*) gene for exfoliation glaucoma in the Icelandic population [119], whereas the first POAG GWAS identified 3 susceptible loci in the Japanese population [120]. Moreover, there are 3 GWAS analyses on PACG, mainly based on the Asian populations [121–123]. Most of the GWAS-identified variants are located in the intergenic region, indicating the possible involvement of the transcriptional regulation on the disease-associated gene expression. In this section, the promoter variants for glaucoma were summarized and discussed.

19.4.1 Myocillin Gene

MYOC on chromosome 1q24.3 is the first disease-causing gene identified for POAG [38]. Its mutations account for 0.3–4.3% of POAG patients [124]. Apart from the mutations in exons, a *MYOC* promoter variant mt.1 (–1000 C/G) is associated with more rapid worsening for both optic disc and visual field measures of glaucoma progression [125]. It is also associated with poor IOP control, greater visual field damage, and a lack of response to therapeutic intervention in POAG patients [126]. However, in our Hong Kong Chinese population, the *MYOC* mt.1 promoter variant is not associated with the risk of POAG [127]. In addition, a meta-analysis showed that another *MYOC* promoter variant rs2075648 is significantly associated with POAG risk in the Caucasian populations, but not in other ethnic populations [128]. These indicate that the association of *MYOC* promoter variants with POAG could be specific in the Caucasian populations.

19.4.2 Cytochrome P450 Family 1 Subfamily B Member 1 Gene

Cytochrome P450 family 1 subfamily B member 1 (*CYP1B1*) gene on chromosome 2p21 was identified as the disease-causing gene for primary congenital glaucoma [129]. Similar to the *MYOC* gene, *CYP1B1* promoter variant rs2567206 (c.-236T>C) has been reported to be associated with primary congenital glaucoma in the Indian population, but not with POAG and PACG [130]. Luciferase assay in the trabecular meshwork cell line showed a 90% reduction in *CYP1B1* promoter activity with the C-allele of rs2567206 variant, compared to the T-allele. However, a meta-analysis of six studies reported no significant association of *CYP1B1* promoter variant rs2567206 with POAG [131].

19.4.3 Caveolin-1 Gene

Caveolin-1 (*CAV1*)/*CAV2* locus on chromosome 7q31.2 was first identified to be associated with POAG in the Icelandic population by GWAS analysis [132]. The most significantly associated variant rs4236601 is located in the promoter region of *CAV1* gene. We confirmed the association of CAV1 rs4236601 variant with POAG in the northern and southern Chinese populations with OR of 5.26; however, this variant is not polymorphic in the Osaka Japanese cohort [133]. In spite of its association with POAG, the genotypes of rs4236601 would not influence the expression and distribution of CAV1 protein in the retinas of donor's eyes from the Caucasian population [134]. Apart from the rs4236601 variant, another variant located upstream of the *CAV1* gene (rs17588172:T>G) was also shown to increase 1.5-fold susceptibility to high tension glaucoma and associated with IOP elevation in the Korean population [135]. It is also associated with early paracentral visual field in POAG patients [136]. The G-allele is associated with the decreased *CAV1* gene expression in skin and adipose by the Genevar eQTL analysis [135]. Coherently, we demonstrated that CAV1-knockout weakens the adhesion of human trabecular meshwork cells and increases the autophagy activity (Wu et al. unpublished data). Collectively, the reduced *CAV1* expression could contribute to the development of POAG.

19.4.4 Cyclin-Dependent Kinase Inhibitor 2B Gene

Cyclin-dependent kinase inhibitor 2B (*CDKN2B*) gene variant (rs1063192) on chromosome 9p21 was first identified to be associated with the vertical cup-disc ratio in a GWAS analysis on the optic disc parameters [137]. In the Australian population, one CpG island (F1:13-14) in the *CDKN2B* promoter showed a significant association with normal tension glaucoma, especially in female subjects [138]. The methylation at the CpG islands in the *CDKN2B* promoter is also associated with genotype at rs1063192, indicating that the expression variation of *CDKN2B* gene could be involved in the development of POAG.

19.4.5 Lysyl Oxidase-Like 1 Antisense RNA 1 Gene

LOXL1 gene on chromosome 15q24.1 is the first GWAS-identified gene for exfoliation glaucoma [119]. Instead of the *LOXL1* gene variant, the variants in the LOXL1 antisense RNA 1 (*LOXL1-AS1*) gene promoter region, the long noncoding RNA encoded on the opposite strand of *LOXL1*, showed strongest association with exfoliation syndrome in the South African population [139]. The *LOXL1-AS1* expression could be changed in response to oxidative stress in human lens epithelial cells and in response to cyclic mechanical stress in human Schlemm's canal endothelial cells. The variants in the *LOXL1-AS1* promoter region could modulate the activity of the *LOXL1-AS1* promoter, which could contribute to the development of exfoliation glaucoma.

19.4.6 Apolipoprotein E Gene

The Alzheimer's disease-associated *APOE* promoter variants were first suggested to be associated with the POAG phenotypes by the candidate gene analysis [140]. The *APOE* promoter variant (c.-219G>T) is associated with the increased cup-to-disk ratio and visual field alteration, whereas the c.-491A>T variant interacts with the *MYOC* promoter variant (-1000 C/G) and is associated with increased IOP and poor response to the IOP-lowering treatments in POAG patients. In the British population, no evidence of association between *APOE* promoter variants c.-219G>T or c.-491A>T and POAG was found [141]. In the Turkish population, although the *APOE* promoter variant (c.-219G>T) showed no significant association with POAG, the POAG patients carrying the GG genotype have higher mean linear cup-to-disc ratio and disease progression, compared to those carrying the GT genotype [142]. Similarly,

in our Hong Kong Chinese population, no significant difference was detected in the frequencies of *APOE* promoter variants between POAG patients and control subjects [143]; yet, the POAG patients with the G-allele of c.-219G>T variant carriers showed a higher age of diagnosis compared to those with the TT genotype. Altogether, these indicate that the *APOE* promoter variants could be a potent modifier for POAG.

19.4.7 Inflammation-Related Genes

The tumor necrosis factor-α (*TNFA*) promoter variant (c.-308G>A) is associated with POAG and pseudoexfoliation glaucoma, but not with chronic PACG in the Iran population [144]. It is also associated with POAG in the Turkish population [145]. However, a meta-analysis of 13 studies revealed no significant association of the *TNFA* c.-308G>A variant with any type of glaucoma [146]. This meta-analysis also showed no significant association of the *TNFA* c.-238G>A variant with glaucoma. Instead, the A-allele of the *TNFA* c.-863C>A variant is lower in POAG patients from the Taiwan population, compared to that in control subjects [147]. Besides, The frequency of (T-allele of *TNFA* c.-857C>T variant and A-allele of optineurin (*OPTN*) c.412G>A variant) or (A-allele of *TNFA* c.-863C>A variant and A-allele of *OPTN* c.603T>A variant) carriers is significantly higher in POAG patients than in control subjects from the Japanese population [148]. These carriers had significantly worse visual field scores than those without *OPTN* variants.

The *IL1A* promoter variant (c.-889C>T) showed an increased risk to POAG in the Taiwan population [149]. The T-allele of the *IL1A* c.-889C>T variant has been shown to increase the expression of IL1A gene. In contrast, the *IL1B* promoter c.-511 is not associated with POAG in the Taiwan population [150]. Besides, the *IL6* promoter variant c.-174G>C has also been reported not to be associated with POAG in the Austrian population [151].

19.4.8 Nitric Oxide Synthase Genes

The endothelial nitric oxide synthase (*NOS3*) promoter variant (c.-690C>T), lying between the cAMP regulatory element (c.-726 to c.-732) and an activator protein-1 binding domain (c.-655 to c.-661), is significantly associated with familial POAG [152]. However, the *NOS3* promoter variant (c.-786T>C) is not associated with POAG in the Taiwan Chinese population [153]. Instead, the normal tension glaucoma patients with CC genotypes of the *NOS3* c.-786T>C variant showed lower mean diastolic and systolic pressure during the day and night in the Poland population [154].

The CCTTT-microsatellite in the inducible nitric oxide synthase (NOS_2) gene promoter showed a significant difference in allele distribution between POAG patients and control subjects in the Sweden population [155]. The (CCTTT)14 allele, which is significantly more abundant in POAG patients, exhibits specific binding of nuclear proteins and a higher reporter activity.

19.4.9 Matrix Metalloproteinase Genes

A meta-analysis of five studies with 1261 glaucoma patients and 1089 control subjects showed a significant association of *MMP1* promoter variant rs1799750 with PACG under homozygous and allelic models and with POAG and exfoliation glaucoma under recessive model [156].

The *MMP2* promoter variants c.-735C>T and c.-1306C>T are not associated with POAG; yet, the TT genotype of both *MMP2* promoter variants are significantly associated with the rim area factor at the early stage of POAG patients from Poland [157].

The *MMP9* promoter variant c.-1562C>T is significantly associated with POAG and PACG under the dominant model in north Indian population [158]. The T-allele of the *MMP9* c.-1562C>T variant confers 1.9-fold higher risk of developing PACG for male patients as compared to the control subjects.

19.4.10 Other Genes

The catalase (*CAT*) promoter variant rs1001179:C>T showed a trend of increase in the visual acuity of PACG patients in the Saudi Arabia population, compared to the control subjects [159].

19.5 Cataract

Cataract remains the leading cause of reversible blindness in developing countries, affecting 95 million people worldwide [160]. Based on the etiology, cataracts can be classified as age-related cataract, pediatric cataract, and secondary cataracts. Age-related cataract is most common in adults, with the onset between age 45 and 50 years. Even with the advancement of technologies and techniques for cataract surgery, the pathogenesis of age-related cataract remains elusive, which is believed to be greatly influenced by the environmental factors. Congenital cataract refers to lens opacity presented at birth, whereas infantile cataract refers to lens opacity developed during the first year of life. Pediatric cataracts have a different pathogenesis than that of age-related cataracts.

Cataract genetic research studies focused on pediatric cataract as one-third of pediatric cataracts are inherited [161]. With the development of whole exome sequencing analysis [162], more than 1000 gene variants have been identified for inherited cataracts in family linkage and candidate gene studies (https://cat-map.wustl.edu/; [163]). Compared to the congenital cataracts, the genetic variants contributing to age-related cataract are largely unknown, which could be complicated by the influences of environmental factors, including sunlight exposure and cigarette smoking [164]. Nevertheless, a recent GWAS analysis on 7050 patients with age-related nuclear cataract identified two loci for nuclear cataract: *KCNAB1* and *CRYAA* [165]. In this section, the promoter variants for cataracts were summarized and discussed.

19.5.1 Crystallin-α A Gene

Crystallin-α A (*CRYAA*) gene, a major protein component of lens, on chromosome 21q22.3 was first identified for the autosomal dominant congenital cataract [166]. A variant (rs11911275) downstream of *CRYAA* gene was also reported to be associated with age-related nuclear cataract in Asian populations, which the downregulation of CRYAA in human lens capsule is correlated with the increase severity of nuclear cataract [165]. In addition to the downstream variant, 2 *CRYAA* promoter variants (rs13053109 and rs7278468) were also reported to be associated with age-related cataract as well as cortical cataract [167]. The rs7278468 variant lies in a consensus binding site for the transcription repressor KLF10, and the T-allele of rs7278468 variant is associated with the increased binding of KLF10 and the inhibition of *CRYAA* transcriptional activity. The epigenetic repression of *CRYAA* gene has been implicated in age-related cataract [168] as well as in high-myopic cataract [169].

19.5.2 Crystallin-γ B Gene

Crystallin-γ B (*CRYGB*) mutation on chromosome 2q33.3 is rare for congenital cataract [170]; yet, the *CRYGB* promoter variant rs2289917 (c.-47T>C), which is predicted binding to ACE2 and progesterone receptor transcription factors, varies significantly among different age groups in the control population of western Indian origin [171]. The C-allele of *CRYGB* rs2289917 variant confers an increase susceptibility to pediatric cataract with OR of 3.34 in the Indian population [172]. In addition, the *CRYGB* rs2289917 variant is also associated with age-related cataract in the Ukrainian population, and the patients with CC genotype of the rs2289917 variant showed higher expression of CRYGB in platelets, compared to those carrying the T-allele [173].

19.5.3 Ferritin Light Chain Gene

Ferritin light chain (*FTL*) gene on chromosome 19q13.33 was discovered for the autosomal dominant trait of hereditary hyperferritinemia-cataract syndrome with a combination of elevated serum ferritin not related to iron overload and congenital nuclear cataract [174]. Point mutations, such as c.-176T>C, c.-171C>G, c.-168G>T, c.-167C>T, and c.-161delC [175–177] were found in the cis-acting element of *FTL* promoter, known as iron regulatory element (IRE). The mutations in the IRE disturb the binding of iron regulatory proteins, leading to an increase in FTL production regardless of the serum iron concentration [178].

19.5.4 Transmembrane Protein 114 Gene

Transmembrane protein 114 (*TMEM114*) gene on chromosome 16p13.2 was discovered as the disease-causing gene for congenital lamellar cataract because of a balanced familial chromosomal translocation t(16;22)(p13.3;q11.2) [179]. The breakpoint lies in the promoter region of *TMEM114* gene and separates this gene from the predicted eye-specific upstream transcription factor binding sites. Further mutation screening in congenital cataract patients identified missense mutations (p.I35T and p.F106L) in *TMEM114* gene, confirming its contribution to congenital cataract. In the mouse lens, Tmem114 expression was found in the lens epithelial cells extending into the transitional zone, possibly involved in early fiber differentiation.

19.5.5 Ras Related GTP Binding A Gene

Ras related GTP binding A (*RRAGA*) gene on chromosome 9p22.1 was discovered to be associated with autosomal dominant juvenile-onset cataract in our Shantou Chinese cohort by whole exome sequencing analysis [180]. In addition to the missense mutation (p.Leu60Arg), we identified a promoter variant (c.-16G>A) of the *RRAGA* gene in a patient with congenital nuclear cataract. This c.-16G>A promoter variant was predicted to abolish a CpG island and a binding site for E2F1, a transcription factor that regulates mechanistic rapamycin complex 1 (mTORC1) signaling. Luciferase reporter assay confirmed that the A-allele of the c.-16G>A promoter variant showed lower transcription activity than the G-allele.

19.5.6 Other Genes

The interferon-γ receptor 1 (*IFNGR1*) promoter variant (c.-56C>T) was reported to be associated with an increased risk of atopic cataracts in the Japanese population [181]. The reporter assay showed that, after stimulation with IFN-γ, the T-allele of the c.-56C>T variant showed higher transcriptional activity of *IFNGR1* gene in lens epithelial cells than the C-allele. Furthermore, higher *IFNGR1* gene expression was found in lens epithelial cells with atopic cataract, compared to that in senile cataracts.

Ephrin receptor A2 (*EPHA2*) gene has been shown to be associated with childhood cataract as well as age-related cataract [182]. A *EPHA2* promoter variant rs6603883, lying in a PAX2 binding site, showed a decreased EPHA2 transcriptional activity in the C-allele, compared to the T-allele, by reducing the binding affinity of PAX2 [183].

Although the catalase (CAT) activity has been shown to be reduced in the plasma of cataract patients than that in the control subjects [184], the *CAT* promoter variant (c.-21A>T) is not significantly associated with age-related cataract in the Chinese population [185]. Coherently, another *CAT* promoter variant c.-262C>T is also not associated with the risk of age-related cataract in the Iran population [186].

19.6 Summary and Future Perspectives

The contribution of promoter variants to the promoter activity and the gene expression is clear and definite. Investigations on the association of promoter variants with complex eye diseases are challenging: (1) Genetic variants exist in a haplotype with strong linkage disequilibrium. It is difficult to identify the causal variant just based on the statistical methods. The localization of the risk and protective variants could provide a hint, which the causal risk variant would not locate in the same allele with another protective variant [64]. Nevertheless, it is still a rate-limiting step for the functional analyses on each variant. (2) Limited studies were reported to study a particular promoter variant with complex eye diseases, and the association of the promoter variants with the complex eye diseases could be population specific. Replication studies in different populations should be conducted to verify the association of each individual promoter variant. (3) Misexpression of a gene is not only caused by the promoter variants with transcription factor binding site changes, but could also be affected by multiple processes, including copy number variation [187] as well as stability and subcellular localization of mRNA and protein [188]. (4) Transcriptomics is a dynamic process. Single variant in the promoter region might not solely contribute to the disease phenotypes. Interactions with other variants or other genes could be possible, but complicated the whole scenario. The retinal cells derived from the induced pluripotent stem cells carry the patients' genome and could mimic the transcriptome of the patients' cells [189]. (5) How the promoter variants can cause the complex eye diseases? Further research is needed to understand the underlying mechanisms of long-term, low-dose aberrant gene expression in the development of complex eye diseases.

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