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REVIEW

Stargardt disease and progress in therapeutic strategies

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ABSTRACT

Background: Stargardt disease (STGD1) is an autosomal recessive retinal dystrophy due to mutations in ABCA4, characterized by subretinal deposition of lipofuscin-like substances and bilateral centrifugal vision loss. Despite the tremendous progress made in the understanding of STGD1, there are no approved treatments to date. This review examines the challenges in the development of an effective STGD1 therapy.
Materials and Methods: A literature review was performed through to June 2021 summarizing the spectrum of retinal phenotypes in STGD1, the molecular biology of ABCA4 protein, the in vivo and in vitro models used to investigate the mechanisms of ABCA4 mutations and current clinical trials.
Results: STGD1 phenotypic variability remains an challenge for clinical trial design and patient selection. Pre-clinical development of therapeutic options has been limited by the lack of animal models reflecting the diverse phenotypic spectrum of STDG1. Patient-derived cell lines have facilitated the characterization of splice mutations but the clinical presentation is not always predicted by the effect of specific mutations on retinoid metabolism in cellular models. Current therapies primarily aim to delay vision loss whilst strategies to restore vision are less well developed.
Conclusions: STGD1 therapy development can be accelerated by a deeper understanding of genotype-phenotype correlations.

Introduction

Stargardt disease (OMIM 248200), also known as STGD1, is an autosomal recessive inherited retinal disease caused by biallelic mutations in the ATP-binding cassette transporter subfamily A4 gene (ABCA4, OMIM *601691) (1–3). STGD1 is one of the most common genetic inherited retinal diseases (IRDs) accounting for 12% of IRD-related blindness (4) and was originally described as a macular dystrophy; however, it is now well recognized that the spectrum of the disease can vary from childhood-onset cone-rod dystrophy with rapidly progressive central and peripheral vision loss, to late-onset macular pattern dystrophy-like disease that tends to spare the fovea (5–7). In this era of translational personalized medicine, a deeper understanding of the genotype-phenotype correlation is essential for appropriate patient selection for the emerging gene- and cell-based therapies. Herein, we review the spectrum of STGD1 in humans and the model systems currently available for investigating pathogenesis and treatment. We then discuss the pathophysiology of gene expression, protein function and consequences of ABCA4 mutations and conclude with current therapeutic options.

Human STGD1 phenotype

Clinical assessment of STGD1

The retinal phenotype in STGD1 depends on the combined severity of the two *ABCA4* mutations, the duration of the disease and other environmental or modifier gene effects. The first attempt to classify disease stages was based on clinical examination (8) (Table 1). As fundus autofluorescence (FAF) imaging enabled better visualization of fleck lesions before they become visible on color photography and delineation of the boundaries of retinal pigment epithelial (RPE) atrophy (9,10) several grading systems based on FAF have also been developed using the 30° or 55° lens of the Heidelberg device (11) and the ultra-widefield Optos camera (12). The relationship between atrophy area on FAF and genotype severity has also been examined (13). Functional classification based on electroretinography (ERG) responses of cone and rod pathways in addition to macular dysfunction has also been developed (14). The value in classifying disease severity based on function is further supported by the demonstration of clinically significant progression in only 22% of the patients from group 1 as compared to 100% from group 3 (14).

Table 1. Classification and staging in *ABCA4*-associated retinopathy according to outcomes of fundus examination, fundus photography, autofluorescence and electrophysiology.

Fundus examination	Topcon fundus camera	Heidelberg FAF	Optos FAF	ISCEV ERG
Fishman 1976 [8]	Fujinami et al. 2015 [15]	McBain et al. 2012 [11]	Klufas et al. 2018 [12]	Lois et al. 2001 [14]
Stage 1: localised central retinal pigment epithelium, photoreceptor and occasionally choriocapillaris disease	**Grade 1:** Normal fundus	**Type 1:** localised low AF signal at the fovea surrounded by a homogenous background with/without perifoveal foci of high or low signal	**Type I:** central atrophy with or without flecks confined to posterior pole—no finding outside 55° field of view	**Group 1:** Abnormal PERG P50 with normal full-field ERG
Stage 2: More diffuse, opaque, partially and totally resorbed flecks	**Grade 2:** Macular and/or peripheral flecks without central atrophy	**Type 2:** Localised low AF signal at the macula surrounded by a heterogeneous background and widespread foci of high or low AF signal extending anterior to the vascular arcades	**Type II:** central atrophy with flecks only extending outside posterior pole	**Group 2:** Abnormal PERG P50 and additional generalised cone ERG abnormality (assessed with light adapted 30 Hz flicker and 3.0 cd·sm^{-2} flash at 2 Hz)
Stage 3: Diffuse, totally resorbed flecks	**Grade 3:** Posterior atrophy **3a:** central atrophy without flecks **3b:** central atrophy with macular and peripheral flecks **3 c:** paracentral atrophy with macular and/or peripheral flecks	**Type 3:** Multiple areas of low AF signal at posterior pole with a heterogeneous background and/or foci of high or low signal	**Type III:** Central atrophy and significant extramacular flecks and atrophy extending outside posterior pole	**Group 3:** Abnormal PERG P50 and additional generalised cone and rod ERG abnormality (the latter assessed using dark adapted 0.01 cd·sm^{-2} and 3.0 cd·sm^{-2} flashes)
Stage 4: Diffuse, totally resorbed flecks and extensive choriocapillaris atrophy	**Grade 4:** Extensive atrophy extending beyond the vascular arcades		**IIIA:** mild/moderate atrophy (most often in punctate pattern) extending to or beyond equator **IIIB:** severe atrophy extending from macula to equator **IIIC:** extensive, severe atrophy extending anterior to equator	

FAF; fundus autofluorescence, ERG; electroretinography, ISCEV; International Society for Clinical Electrophysiology of Vision

Multimodal imaging and functional assessment are required in the baseline assessment of a patient with suspected STGD1. If possible, it is highly recommended that all immediate family members are also examined to identify late-onset STGD1 without symptoms or masquerading as age-related macular degeneration, and pedigrees with pseudodominant inheritance (16). While the purpose of clinical assessment in STGD1 is to guide genetic analysis and variant interpretation, it is also important to document biomarkers of disease progression and prognosis. Whether STGD1 presents as a pure macular dystrophy, cone dystrophy or cone-rod dystrophy, this condition is uniquely characterised by the excessive deposition of lipofuscin in the RPE followed by progressive RPE and outer retinal loss. The following investigations are recommended.

Clinical examination

The best-corrected visual acuity should be recorded as it provides an indication of foveal sparing. Colour vision assessment is useful for differentiating STGD1 from phenotypically similar cone dysfunction syndromes. Fundoscopy typically reveals retinal flecks around the macular region with or without a mid-peripheral distribution. However, by the time, a beaten bronze sign appears, the vision loss is already profound. Large peripheral pigmented lesions and even extensive bone spicules can be seen in those with childhood-onset disease (17).

Multimodal retinal imaging

Multimodal imaging should include spectral domain optical coherence tomography (SD-OCT), FAF and ultra-widefield (UWF) imaging. OCT thickness and angiographic parameters were shown to be statistically different between 70 STGD1 patients as compared to 70 healthy eyes (18). Therefore, SD-OCT should be performed to assess the degree and extent of outer retinal loss and RPE atrophy. OCT macular volume decline can serve as a biomarker for disease progression (19). However, automated segmentation of the diseased RPE-Bruch's membrane is unreliable and requires manual adjustment using an "adaptive" approach or automated deep learning segmentation (20,21). Flecks are best visualized on FAF and their growth and life cycle may serve as a biomarker for disease progression where one study has developed deep learning segmentation of flecks in STGD1 (22). FAF imaging can also illustrate early macular RPE atrophy. Recent studies have demonstrated differences in atrophy between FAF images generated by near-infrared versus short-wavelength excitation light (23–25). Near-infrared FAF has been recommended as an adjunct to short-wavelength FAF given the earlier detection of hypoautofluorescent lesions with excellent intra- and inter-observer reliability of areas of decreased FAF. UWF retinal imaging, in both colour and AF modes, should be used in all cases as it allows for the visualization of the peripheral retina and is useful particularly in the assessment and monitoring of childhood-onset disease. Severity of UWF-FAF has been classified into three types depending on the extent of peripheral retinal involvement and atrophy (12). Each of these three phenotypes was associated with increasing OCT severity.

Functional assessment

In addition to structural assessment, objective functional evaluation with electrophysiology is useful for disease monitoring, determining prognosis and patient counselling (26). Of those with a normal full-field ERG at baseline only 20% showed significant progression after 10 years of follow up. Full-field ERG findings have been used to classify STGD1 into mild and severe phenotypes (27) whilst ERG features have also been used to classify *ABCA4* mutation severity (28). Microperimetry has been used do document central visual field impairment in STGD1 (29). It is being investigated as a clinical trial endpoint but its role in routine monitoring of STGD1 progression remains uncertain (30). The measurement of total volume beneath the sensitivity surface of a 3-D model of the hill of vision derived from microperimetry outputs has been proposed as an alternative to average sensitivity decline (31). Unfortunately, microperimetry remains challenging or impossible to perform in those with advanced disease and a preferred retinal locus outside the posterior pole. Widefield perimetry, using the Octopus or the Goldmann device, maybe the only method to measure residual peripheral vision in STDG1 with severe posterior pole disease (32). However, caution is required in interpreting results in children as there is large test–retest variability and the area of intact peripheral field has been shown to increase substantially as these children age (33).

Clinical presentation of STGD1

Following the identification of the causative gene (1,7,34–39), several large case series of genetically confirmed STGD1 have reported a wide spectrum in the age of presentation and clinical manifestations. These can be divided into childhood-onset, early adult-onset and late adult-onset. Each of these groups may have their own variations in phenotype that correlate with specific *ABCA4* variants. The variability in age of onset can also be found within the same family (16,40).

Childhood-onset *STGD1*

Children with biallelic severe or null-like *ABCA4* variants present with central visual loss attributed to macular dysfunction alone or in combination with cone-rod dystrophy (CORD3, OMIM 604116) between the ages of five and eleven years (Figure 1). The diagnosis of childhood-onset STGD1 may be challenging in the early stages of the disease when the fundus may appear normal and the full-field ERG may be unremarkable resulting in delayed diagnosis (41). Up to 24% of children less than 10 years of age have shown a normal fundus appearance (41). Thus, the absence of flecks or macular atrophy early in the disease is a relatively common finding and examination may not identify lesions until 3 years after the onset of symptoms, with the earliest signs being retinal pigment epithelial (RPE) alteration, bull's eye appearance and parafoveal flecks. Therefore, children may initially be labelled with functional vision loss or treated for amblyopia (42). This misdiagnosis has led to a median delay of 3 years in 50–90% of cases (41,42). However, given the widespread accessibility and use of OCT to detect early features of STGD1, the frequency of misdiagnosis is expected to decline.
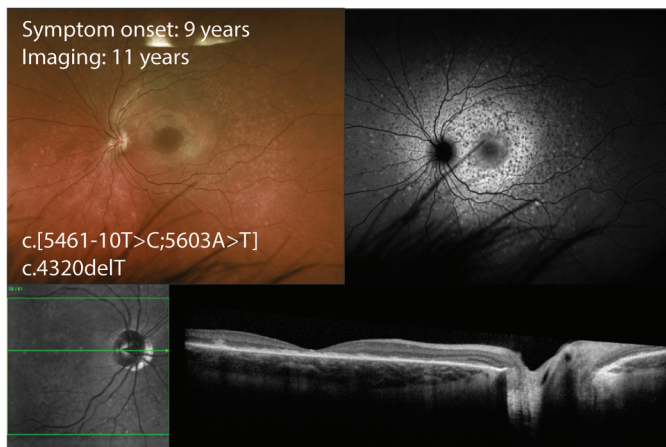
Figure 1. Wide-field colour and autofluorescence imaging in an eleven-year-old with childhood-onset STGD1. The two alleles are effectively null. There is increased autofluorescence signal in the posterior pole with hyperautofluorescent flecks and speckled hypoautofluorescence. Optical coherence tomography demonstrates subretinal debris and outer retinal layer loss in the foveal region.

In children with no abnormalities on clinical examination, FAF has demonstrated discrete hyperautofluorescent dots confined to the foveola and even mild hyperautofluorescence in the perifoveal region (43). Furthermore, a broader and more diffuse band of hyperautofluorescence with increasing retinal eccentricity has also been demonstrated (43). FAF abnormalities have been reported in most patients with normal fundoscopy (43). A thickened external limiting membrane (ELM) as seen on SD-OCT may provide an early biomarker for childhood-onset disease (43,44). This thickening demonstrated a maximal prominence at the foveola and decreased symmetrically with increasing eccentricity. These changes may represent disruption of the outer nuclear layer (ONL) within the cones and this is consistent with cone photoreceptor nuclei residing close to the ELM in the perifoveal region (45). Sequential OCT images have illustrated focal collapse of the inner retinal layers, secondary to loss of the outer retinal structures (43). These changes appear to preferentially affect perifoveal areas as the foveola photoreceptors are more resistant to degenerative processes (43). Intraretinal pigmentations have been correlated to hyperreflective deposits in the inner layers of the fovea.

In childhood-onset STGD1, there may be a normal or near-normal pan-retinal cone and rod function on ERG however many of these patients later develop abnormal full-field ERG amplitudes (3,14,41,15,46). Normal full-field ERG findings have been reported in 74% of children, with no obvious fundus abnormalities, at an average of two years (range, 0.1–27) after the onset of vision loss (42). In this study, group 3 ERG recordings (Table 1) were obtained in 11% of patients at 18 years (range, 6–27) after the onset of disease (42). When assessing the five, an abnormal pattern ERG P50 component has been reported in children with no evidence of macular atrophy illustrating macular dysfunction (43). Despite having a normal peripheral retina on fundoscopy four children have demonstrated generalized retinal dysfunction on full-field ERG (43).

A form of rapid-onset chorioretinopathy (ROC), was described in a third of cases with biallelic null or severe

ABCA4 mutations where the mean age of onset was seven years, and the key features were early and severe vision loss (20/200 to counting fingers), group 3 ERG findings, extensive bone spicules with attenuated retinal vasculature and markedly increased AF signal (47).

A controversial manifestation is retinitis pigmentosa (RP19, OMIM 601718) (48–53). It is now accepted that the RP phenotype refers to heavy pigmentation seen in the later stages of a rapidly progressive CORD3 phenotype. Close examination of "typical" RP reveals extensive macular and choroidal atrophy, with peripheral pigmentation (48,51,53). These cases had early central visual loss that rapidly progressed to peripheral field loss and night vision impairment. This is probably the same as ROC (47), except the phenotype was documented at a later stage when fundus features were dominated by peripheral atrophy, pigment plaques and bone spicules (50). Such severe cases of "inverse" RP or ROC are generally associated with two null or deleterious variants with a low basal ATPase activity not stimulated by N-retinylidene-phosphatidylethanolamine (54).

Early adult-onset STGD1

These patients present with central visual loss due to foveal atrophy in their second or third decade of life. At presentation, fundoscopy typically shows prominent macular atrophy with numerous paramacular and peripheral flecks. A key clinical finding is also peripapillary sparing (55), although this is not specific. These patients often carry *ABCA4* variants with an intermediate impact on ABCA4 function (e.g. c.6079C>T and c.[2588G>C;5603A>T]) either on both alleles or as compound heterozygous combination with a null/severe *ABCA4* variant. In contrast to childhood-onset disease, their retinal flecks tend to spread centrifugally to cover most of the retina whilst the atrophy expands peripherally to only the perimacular region by the 5th decade of life (56,57). An exception is a subset of patients carrying the c.5882G>A, p.(Gly1961 Glu) variant who also develop symptoms in early adulthood (Figure 2).

The phenotype linked to the c.5882G>A, p.(Gly1961Glu) variant (5,58–63) is bull's eye maculopathy with paucity of flecks and normal full-field ERG (Figure 2). The p. (Gly1961Glu) phenotype typically demonstrates a Fishman clinical grading of I or occasionally II, FAF imaging has shown a small region of decreased AF in the fovea, with or without a continuous ring of increased AF and all cases have normal dark and light-adapted full-field ERG. Those with homozygous p.(Gly1961Glu) complexed with another missense variant (His1838Asp or Asn96Lys) had childhood onset disease (4–12 years at onset) with Fishman clinical grading of III or IV and ERG group III (62). Those with a deleterious mutation *in trans* with p.(Gly1961Glu) tended to have an earlier onset however the range was wide (13,28). A multicenter study of 79 cases carrying this mutation showed a greater penetrance of this gene in females, but no age difference in onset (64). At presentation around a third may have a history of nyctalopia and another third have photoaversion (60). Visual impairment was mild at around 0.8–0.9 in logarithm of minimum angle of resolution (logMAR) scale. Those presenting at a later age may have a larger area of RPE atrophy resembling geographic atrophy (62). Quantitative FAF measurement has shown signal intensity in the 7°-9° zone falling
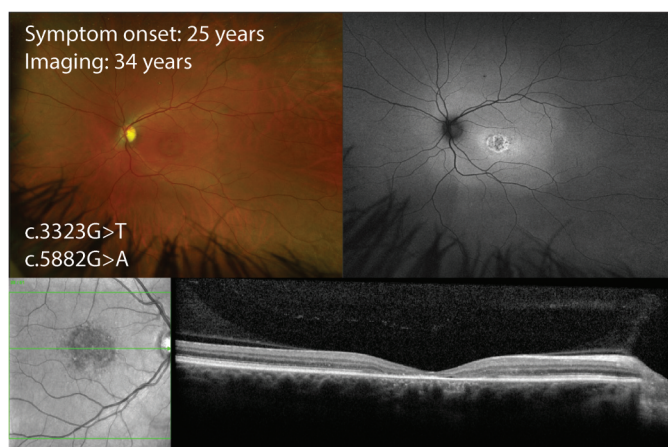
Figure 2. Wide-field color and autofluorescence imaging in a 34-year-old with early adult-onset STGD1 due to an allele with intermediate severity p.(Arg1108Leu) and the common variant p.(Gly1961Glu) with mild severity. There is a ring of hyperautofluorescence in the fovea with no lesions elsewhere in the retina. Optical coherence tomography demonstrates loss of photoreceptor in the fovea without any signs of fleck deposit.

within the 95% confidence interval for healthy eyes (63). OCT typically shows localized outer segment loss resulting in an optical gap (65,66), but later onset disease may be accompanied by flecks. Microperimetry confirmed a localized foveal or perifoveal scotoma (61,62).

Late adult-onset *STGD1*

The definition of a "late" onset disease has varied from those ≥ 35 to those ≥ 50 years of age (2,67–70). Thus, patients can present from the fourth decade onwards. In contrast to childhood-onset, those with late adult-onset disease are often identified incidentally on retinal screening of family members of an affected individual, or when they become symptomatic secondary to subfoveal choroidal neovascularization. This lack of symptoms is explained by atrophy sparing the fovea. The mean age for onset of symptoms was 55 years (range 45–72 years) in one case series (68). Although clinical examination may only show paracentral atrophy, FAF imaging often shows subtle abnormalities that may spread peripherally (71). Hyperautofluorescent flecks are generally found surrounding the atrophy but they may extend nasally to the disc and peripherally. OCT features are marked by prominent RPE loss with co-localized outer retinal layer loss. Some patients may be misdiagnosed as having age-related macular degeneration if OCT and FAF features of flecks were not recognized at presentation.

The recognition of c.5603A>T p.(Asn1868Ile) as a hypomorphic allele has facilitated the resolution of diagnosis in 80% of cases that were previously labelled as mono-allelic (Figure 3) (72). Patients with Asn1868Ile *in trans* with a severe or deleterious allele had a mean age of symptom onset of 40 years, whilst those with a mild-to-moderate allele may not manifest the disease. There is also increased penetrance in females (64). Those with an age of symptom onset < 30 years old tend to have non-specific visual complaints such as photoaversion and mild nyctalopia (73). The mean visual acuity in the better-seeing eye at presentation was around 0.10 in logMAR (73). FAF typically shows a paracentral atrophy

sparing the fovea surrounded by a hyperautofluorescent zone or discrete fleck lesions without extension beyond the vascular arcades. In pre-atrophic cases the OCT may show increased reflectivity of the ELM band in the perifoveal region sparing the fovea.

Variations in disease progression

The ability to predict disease progression based on genotype and the presenting phenotype is critical for both counselling and the design of clinical trials. Measurement of disease progression rates can be made by calculating the expansion rate of the area of RPE atrophy in near-infrared and short-wavelength FAF images (23,24,74,75), intensity of autofluorescence signal in regions unaffected by flecks or RPE loss (76) and counting of hyperautofluorescent flecks (22,77). The area of definitely decreased autofluorescence was shown to decline by 0.51 (95% confidence interval, 0.42–0.61) mm^2 per year (75). However, area growth is highly dependent on baseline lesion size. A recent study reported a mean expansion rate of 0.69, 0.78 and 0.40 mm^2 per year for children, adults with childhood-onset and adults with late-onset disease (78). Growth rate based on square root transformed area of atrophy has been shown to be independent of baseline area (79). This approach has allowed demonstration of the importance of ERG severity and genotype group on atrophy expansion rates in STGD1 (56,80). Future clinical trials should consider the use of square root transformed parameters in measuring area growth in STGD1 (81). The feasibility of measuring retinal atrophy progression rate by calculating total macular volume decline (19) on volumetric OCT has been described. However, the importance of genotype on the rate of macular volume loss has yet to be investigated. Mapping functional decline based on microperimetry has also been described over a one-year duration in the ProgStar cohort, although the decline was only 0.68 (95% confidence interval, 0.47 to 0.89) dB per year (30). There was no association between microperimetry progression rate and genotype (31).
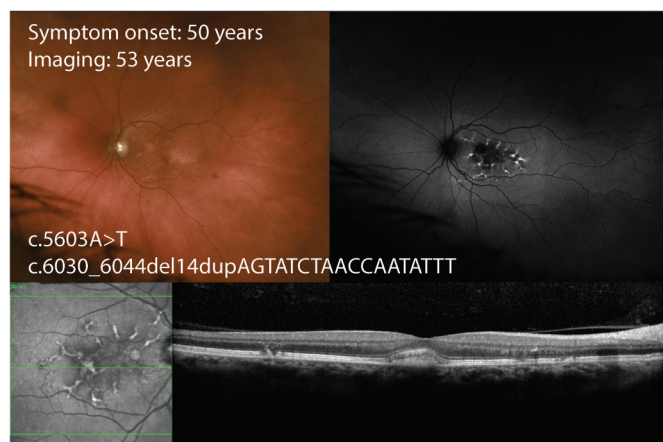

Figure 3. Wide-field color and autofluorescence imaging in a 53-year-old with late adult-onset STGD1 due to a truncating allele and the common hypomorphic variant p.(Asn1868Ile). There are linear branching hyperautofluorescent flecks in the perifoveal region with no lesions outside the macula. Optical coherence tomography demonstrates subretinal deposit resembling a vitelliform lesion and intraretinal migration of the perifoveal flecks.

Molecular biology of the human *ABCA4* gene and protein

The ABCA4 gene

Several groups refined the *ABCA4* gene location to a 2–3 cM interval (36,37). Allikmets et al. localized two markers that flank the *ABCA4* gene, D1S3361 and D1S236, to 1p22.3–1p22.2, therefore repositioning the gene to 1p22 (82). By fluorescence *in situ* hybridization, the chromosomal position of the human *ABCA4* gene was mapped to 1p21-p22.1 (83), the current location published on the National Centre for Biotechnology Information website (NCBI, https://www.ncbi.nlm.nih.gov/nuccore/NM_000350.3). The coding region of the *ABCA4* gene consists of 50 exons, spanning over 100 kb (39,82). According to Ensembl (http://www.ensembl.org), the *ABCA4* exons range in size from 33 bp to 266 bp (Figure 4). There are 8 splice variants for *ABCA4*, three of which are protein-coding transcripts whilst the remaining are non-protein-coding. The full length, annotated *ABCA4* transcript of 7328 bp, consisting of an open reading frame of 6822 bp encoding a 2273-amino-acid protein is published by NCBI (NP_000341.2).

The ABCA4 protein

The ABCA4 protein is localized to the membrane and expressed in the outer segments of photoreceptors. ABCA4 is subclassed under the ABC transporter superfamily, the largest and most diverse family of transmembrane transport proteins that typically utilize the energy of ATP hydrolysis to pump various substrates across cell membranes against concentration gradients. Consistent with other ABC transporters, ABCA4 protein is composed of the typical structure of four domains

for functionality: two highly conserved nucleotide-binding domains (NBDs) that provide energy for translocating substrates by binding and hydrolyzing ATP to ADP, and two highly hydrophobic transmembrane domains (TMDs) that act synergistically with NBDs (Figure 5).

Opinions regarding the function of both NBDs differ. The complementary hydrogen/deuterium exchange studies suggested that only NBD2 is catalytically active (84). This hypothesis is in line with other biochemical studies indicating that NBD2 binds and hydrolyses ATP in the presence or absence of substrates, whereas NBD1, containing a bound ADP, associates with NBD2 to play a non-catalytic role in substrate translocation (85). However, this is not consistent with the results of a mutagenesis study, showing that both NDBs are catalytically active, albeit with different functions; mutations in NBD1, either alone or in combination with mutations in NBD2, abolished both basal and retinaldehyde-stimulated ATPase activity, whereas mutations in NBD2 result in inhibition of ATP hydrolysis stimulated by retinaldehyde (86).

The signature motif of the prokaryotic ABC importers "EAA" was found in the N-terminal transmembrane domain (TMD) of ABCA4, revealing its identity as the only known mammalian importer (87), flipping substrates from the luminal (topographically equivalent to the extracellular) to the cytoplasmic side. However, the "EAA" sequence, which interfaces the NBD and the TMD, is absent in the C-terminal half (88). Two large exocytoplasmic domains (ECD1 with 600 residues and ECD2 with 300 residues) locate inside the disc lumen (84,89). The number of N-glycosylation sites on ECDs are controversial and the biological role of the two large ECDs remains to be determined (90,91). Discussion on the detailed functional and structural characterization of ABCA4 domains are beyond the scope of this review. We refer readers to
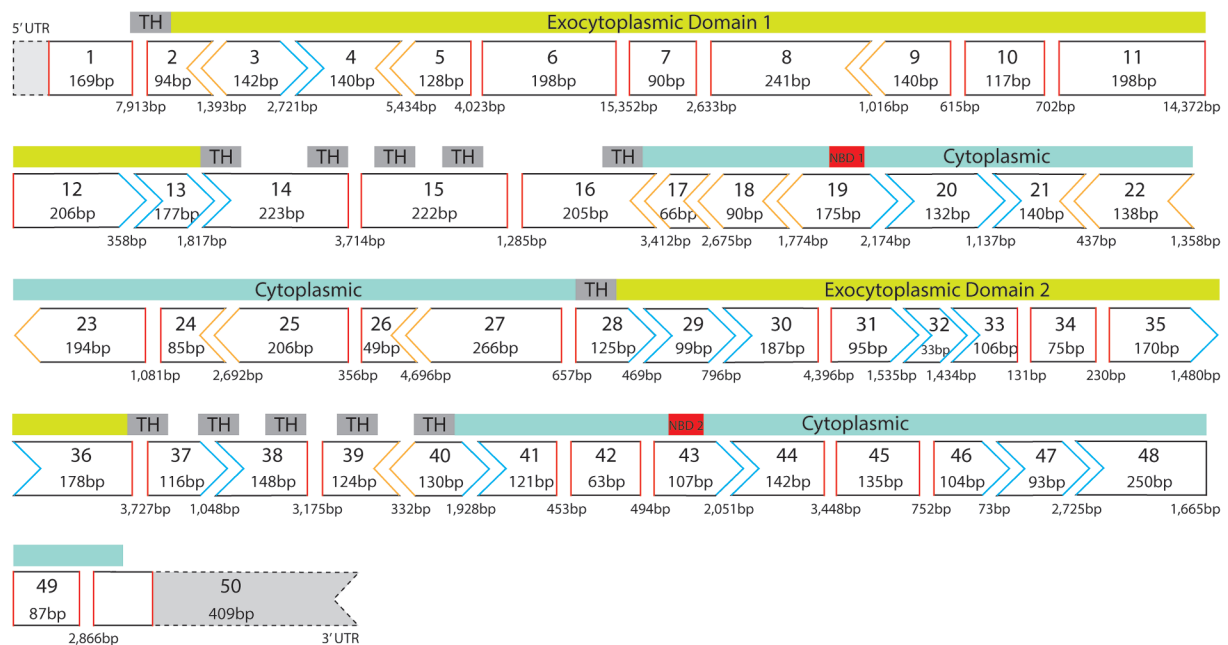
Figure 4. The *ABCA4* exon map, showing the reading frame and the functional protein domains encoded by the corresponding exons. Exon 2–13 and exon 28–36: exocytoplasmic domains, exon 16–28 and exon 40–50: cytoplasmic domains, exon 19 and exon 43: encode nucleotide-binding domains (NBDs). In-frame exons are indicated by rectangle with red sides, whereas codons disrupted by exon junctions are indicated by chevron sides in blue or orange. TH: transmembrane helix; UTR: untranslated region.

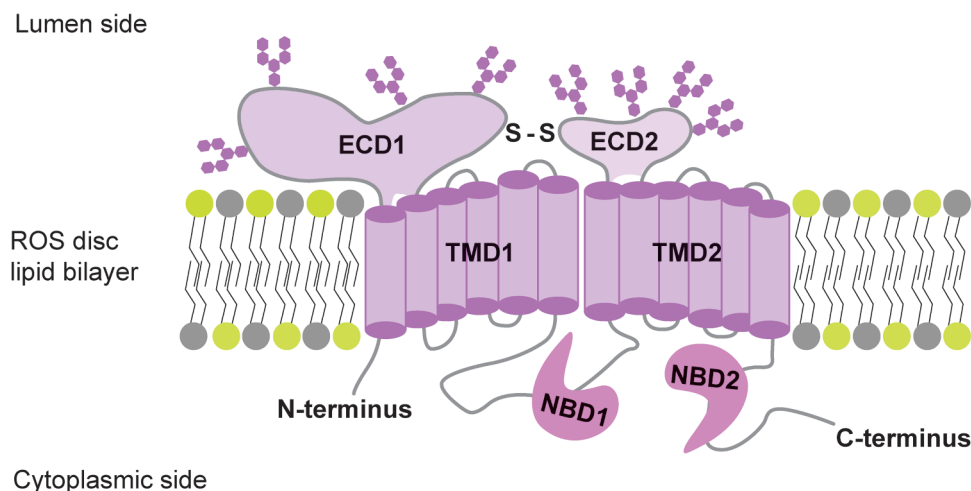**Figure 5.** Topology of ABCA4 protein, adapted from 145. ECD: exocytoplasmic domain; TMD: transmembrane domain; S-S: disulfide bond; NBD: nucleotide-binding domain; ATP: adenosine triphosphate; ADP: adenosine diphosphate; ROS: rod outer segment.

comprehensive reports for more information regarding this aspect (84,88,92–98).

ABCA4 function in the retinoid cycle

Upon photon absorption in photoreceptors, the excessively released 11-*cis*-retinal and its derivatives all-*trans*-retinal rapidly and reversibly react with phosphatidylethanolamine (PE) to form N-retinylidene-phosphatidylethanolamine (N-ret-PE). Depending on the orientation of the retinylidene-bearing head, two forms of N-ret-PE have been proposed: cytoplasmic oriented N-ret-PE and luminal N-ret-PE (99). The cytoplasmic oriented N-ret-PE can be catalyzed by the all-*trans*-retinol dehydrogenases (all-*trans*-RDHs) that exist in the cytoplasm (100,101), producing all-*trans*-retinol products that re-enters the retinoid cycle. However, the luminal N-ret-PE is unable to cross the disc membrane independently (102) and thus is inaccessible by RDHs. By utilizing ATP hydrolysis as an energy source, ABCA4 assists in the elimination of cytotoxic retinoid chemicals through actively flipping the luminal N-ret-PE to the cytoplasmic surface of disk membranes (87,103). A portion of luminal N-ret-PE that has not been flipped by ABCA4 can be further condensed with another all-*trans*-retinal to produce bisretinoids in the discs. Upon phagocytosis by RPE, the condensed compounds in the outer segments of photoreceptors are thought to be eventually converted to bis-retinoid N-retinylidene-N-retinylethanolamine (A2E), the major form of lipofuscin deposits in the low-pH environment of RPE phagolysosomes (102). Recently, it was proposed that ABCA4 performs a similar function in the RPE endolysosomal membrane to that in photoreceptor outer segments, that is, it translocates N-ret-PE from the luminal to the cytoplasmic surface in an ATP-dependent manner (99).

Molecular pathogenesis of ABCA4-STGD1

Mutation spectrum

With the advent of high-throughput next-generation sequencing, an ever-increasing number of *ABCA4* variants have been consecutively reported since the linkage of STGD1 to *ABCA4* in 1997 (1). At the time of submission, a total of 1196 unique *ABCA4* variants have been recorded in the Leiden Open Variation Database (LOVD, https://databases.lovd.nl/shared/genes/ABCA4). Cornelis et al. (104) analyzed 913 unique *ABCA4* variants published before 2016 and concluded that the majority are missense variants (51.59%), followed by protein-truncating variants (34.39%), complex alleles (7%), non-canonical splice site variants (3.61%), variants of non-truncating insertion/deletion (1.75%), deep-intronic variants (1.1%) and synonymous variants (0.55%). Among these, c.5882G>A p.(Gly1961Glu), c.[2588G>C;5603A>T] p. [(Gly863Ala,Gly863del;Asn1868Ile)] and c.[5461–10T>C;5603A>T] p.[Thr1821Aspfs*6,Thr1821Valfs*13;(Asn1868Ile)] are the three most frequent *ABCA4* variants.

Given that *ABCA4* mutations are extremely heterogeneous and several common variants are clustered in specific ethnic groups (105,106), correct interpretation and a consensus on the pathogenic classification of *ABCA4* variants is essential for disease prognosis and potential therapeutic options. Recently, the American College of Medical Genetics and Genomics (ACMG) published a "gold standard" to standardize terminology and scoring system of variants identified in genes that cause Mendelian disorder (107). A five-tier terminology system using the terms "pathogenic," "likely pathogenic," "uncertain significance," "likely benign," and "benign" is recommended based on the classification criteria provided in this report. By using this guideline, pathogenicity and its classification could be assigned to 1103 *ABCA4* variants from LOVD, with 255 variants identified as pathogenic, 158 as likely pathogenic, 94 as uncertain significance, 31 as likely benign and 88 as benign. As currently developing functional assays are rapidly and constantly contributing to an update of variants in this regard, we reviewed pathogenic evidence obtained from these assays in below, for a better understanding of the molecular pathogenesis of certain variants that lead to disease.

Effect of causal missense variants

Over the decades, a series of research methods have been established to understand the effects of *ABCA4* mutations on its function: using fluorescent microscopy and *in situ*

hybridization to characterize cellular localization (99), transiently expressing wild and mutant human *ABCA4* cDNA into cell lines for soluble protein quantification (54,108), *in vitro* measurement of ATPase activities and retinoid substrate-binding activity (87,109–112) for assessing individual functional domain activities etc. Several studies investigated the effects of single amino acid substitution (86,108,113), small in-frame deletions (86,113) and frameshift mutations (86). Some of the mutations resulted in reduced protein levels (86,108), whereas others were found to compromise ATPase activity (113,114) and/or retinoid binding affinity (108,113).

As the major group of variants, missense variants directly compromise the function of protein, or alter the secondary structure that is crucial for correct localization, resulting in functional defects. For example, the amino acid change of Gly863Ala due to variant c.2588G>C, the most common mutation observed in STGD1 patients, attenuated the ATPase and CTPase activities of NBD1 and the rate of ATP hydrolysis significantly, as compared with the normal or WT NBD1 (114). Missense mutation in cysteine residues within ECDs (Cys54Tyr, Cys75Gly, Cys1488Arg, Cys1490Tyr) that alter disulfide bonds, could cause ABCA4 protein to be misfolded and lead to STGD1 (90). Mutations involving glycosylation sites also have impacts on protein structure thus causing diseases. One example is the variant p.Ser100Pro, a STDG1-related mutation that introduces a proline residue and prevents the occurrence of N-linked glycosylation on Asn-98 site (91,115). In this case, the mislocalization or incorrect insertion of ABCA4 protein contributes to photoreceptor degeneration. The retention of mutant ABCA4 protein in the inner segment of photoreceptors, likely due to misfolding, has also been reported in patients with retinal dystrophies (116).

While the function of misfolded protein is compromised, it could overload the cellular processing systems, the capability of which becomes more tenuous under the elevated stress caused by lipofuscin accumulation in photoreceptor and RPE cells. Moreover, such misfolded protein could in turn exacerbate the primary loss-of-function consequence by toxic gain-of-function (115), manifested by apoptotic signaling activation resulting from endoplasmic reticulum stress (117) and unfolded protein response (118). Patients with two non-truncating mutations presenting with greater disease severity than patients carrying two truncating mutations could be explained by such gain-of-function toxicity (115). However, STGD1 remains a recessive disease despite the potential for toxic-gain of function in a carrier of such mutations.

Effect of causal splice variants

Alternative splicing has been considered to contribute to extensive transcriptomic and proteomic complexity by generating multiple transcripts from a defined genomic repertoires, and underlies significant phenotypic difference (119,120). It was estimated that in the retina, 13% of novel mRNA junctions were expressed at levels similar to, or higher than the reference transcripts (121). However, this complexity comes at a cost (122). It is now evident that missense, nonsense or even silent mutations can cause disease through effects on splicing, rather than directly through amino acid changes that impair protein

function (123), and for certain genes these types of mutation can be found in as many as 50% of the cases (123). To characterize the functional consequences of *ABCA4* splice variants, series of *in vitro* splice assays, including midigene assays (124), patient-derived fibroblast-based assays (16), assays employing the induced pluripotent stem cell (iPSC) (125–128) and iPSC-derived retinal cells (127,129,130) etc., have been developed successively.

Mutations affecting the invariant GT or AG sequences at the 5′ (donor) or 3′ (acceptor) of exons typically result in exon skipping (121) due to inactivation of the canonical splice sites, although intron retention has also been reported in some cases. According to data from our laboratory, the mutation c.5835+1G>A affecting the first highly conserved "G" of the *ABCA4* intron 41 disrupts the canonical splice site and leads to the retention of 12 nucleotides downstream of exon 41 and use of a cryptic donor splice site.

Recent analysis of a splice variant derived from our STGD1 cohort revealed that single or multiple exon skipping and intron retention was observed due to the variant location in the noncanonical splice sequence (see definition (124)). One example is the most prevalent variant c.[5461–10T>C;5603A>T]. Patient-derived fibroblast harboring this variant show defective *ABCA4* mRNA splicing, leading to reduced abundance of full-length transcripts and generation of alternatively spliced transcripts missing exon 39 or both exon 39 and 40. Such splicing defects were also validated in patient-derived progenitor cells (127). However, other splice events such as partial exon skipping and exon elongation through intron retention were also reported for noncanonical splice variants (131).

In recent years, mutations located in noncoding regions of *ABCA4*, especially deep intronic mutations are gaining more attention as they account for the missing inheritability in some cases where only monoallelic *ABCA4* variant was detected (121,132–135). Indeed, STGD1 cases where the second allele was later identified as having a deep intronic mutation, aberrant splicing occurred due to the activation of cryptic splice sites or alteration to splice enhancer or silencer motifs. One example is the presence of two neighboring intronic mutations in intron 30 of *ABCA4;* (c.4539+2001G>A and c.4539+2028C>T) created an exonic splicing enhancer and resulted in a retina-specific transcript containing a 345-nt pseudoexon (129). The absence of the aberrantly spliced transcript in patient-derived fibroblasts further implies that the pre-mRNA splicing in the retina is modulated under more complex splicing programs than in other tissues (136), and certain splicing motifs can only be recognized by tissue-specific factors present exclusively in retinal cells (135).

A significant proportion of missense, nonsense, insertion and deletion mutations may in fact exert their effects through altered splicing due to the disruption or introduction of exonic splicing enhancers and silencers or creation of novel splice sites (16,137), highlighting the necessity of validating the presumed effects of pathogenic variants on pre-mRNA splicing.

Impact of altered retinoid metabolism on cellular function and death

As stated above, in the well-studied retinoid cycle (Figure 6) (138), the chromophore of visual pigments consists of 11-*cis*-retinal, the regeneration and the replenishment of which is
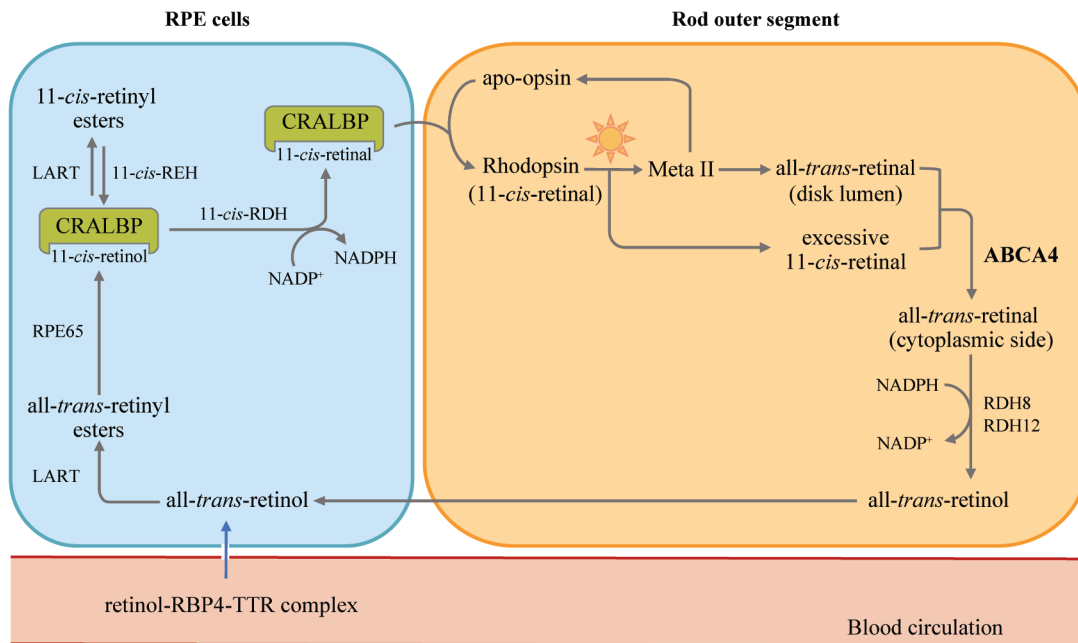
Figure 6. A schematic showing the retinoid cycle. The chromophore (rhodopsin in this figure) consists of 11-*cis*-retinal and undergoes photoisomerization to all-*trans*-retinal. The role of ABCA4 is to actively flip N-retinylidene-phosphatidylethanolamine from the luminal side to cytoplasmic surface, where it reduced by the all-*trans*-retinol dehydrogenases (RDH) to all-*trans*-retinol. Together with that derived from the blood circulation, all-*trans*-retinol enters the RPE and is esterified by LART (lecithin: retinol acyltransferase) to generate all-*trans* retinyl esters. The retinyl ester can either be stored in the RPE cells or used as the substrate for the isomerohydrolase RPE65 to produce 11-*cis*-retinol, which can be later oxidized to 11-*cis*-retinal by NADP+, catalyzed by 11-*cis*-RDH. The 11-*cis*-retinal re-enters the outer segment of photoreceptors, where it assembles with opsin and regenerates rhodopsin. NADPH: nicotinamide adenine dinucleotide phosphate; RBP: retinoid-binding protein; TTR: transthyretin; RPE: retinal pigment epithelium; CRALBP: cellular retinol-binding protein.

mediated by the adjacent RPE cells. However, these elaborate pathways for pigment regeneration also carry potential risks, particularly when various enzymes, retinoid-binding protein and complex diffusional processes are involved.

With the loss of ABCA4 transporter, the transportation and clearance of N-ret-PE is delayed and favors condensation between N-ret-PEs to generate bis-retinoids, such as N-retinylidene-phosphatidylethanolamine (APE), dihydro-N-retinylidene-N-retinylphosphatidylethanolamine (A2PE-H2) and its oxidized form (A2PE) (99). By acid hydrolysis in RPE cells, non-degradable A2E is formed and progressively accumulated as lipofuscin deposits, either within the subretinal space or in the RPE cells (102,139).

Several studies have investigated the mechanisms of A2PE/A2E cytotoxicity. A2PE can serve as photosensitizer and itself can be photo-oxidized, contributing to photo-oxidative damage to photoreceptors (140). A2E not only delays digestion of the lipid components of phagocytosed outer segments thus increasing the undigested substances in RPE (141), it also increases the cytotoxicity of blue light (142,143). A highly reactive form of oxygen, superoxide, can be generated by irradiation of A2E, contributing to the further oxidation of A2E, resulting in photooxidative damage (144). A recent study furthermore indicated that bis-retinoid compound accumulation has a role in complement system activation (145). As a result, the nondegradable A2E fluorophores severely retard RPE phagocytotic and lysosomal functions, subsequently leading to RPE degeneration and loss of overlying photoreceptors.

Animal models developed to study the underlying pathogenesis of STGD1 have been available for some time (Table 2). However, differences of anatomy and disease phenotype

between the mouse and human eye exist, and two important pathological features of STGD1 in humans, the accumulation of A2E in RPE cells and delayed dark adaptation, were observed in $Abca4^{-/-}$ animal models (146–154). Other pathological features, including photoreceptor atrophy and RPE disturbance (147), fundus autofluorescence (147,148) and relevant topical changes eg. oxidative stress response and compliment activation (150–154) are also reproducible in animal models. Of note, the correlation between increased lipofuscin, mainly A2E and partly all-*trans*-retinal dimers (155), and the increased intensity of fundus autofluorescence has been confirmed in $Abca4^{-/-}$ mice (147,156).

The accumulation of all-trans-retinal condensation products followed by accentuated RPE and photoreceptor degeneration at an early age were also observed in the dual $Abca4$ and $Rdh8$ knockout mouse model (157). Moreover, the acute and light-induced retinopathy induced in the $Abca4^{-/-} Rdh8^{-/-}$ mouse model indicates that the free all-*trans*-retinal rather than A2E condensation products appears to be the pathogenic factor in the retinopathy (158). Consistently, Yu et al. found that under exposure of bright light, the increased all-trans-retinal detected in $Abca4^{-/-} Rdh8^{-/-}$ mouse can induce rapid NADPH oxidase-mediated overproduction of intracellular reactive oxygen species (159) that is involved in retinal dystrophy (160).

Given that the macula is primarily affected in STGD1 patients, and mouse models are unable to fully recapitulate human STGD1 disease due to the lack of macula, recent dog models have bridged this gap. The Labrador retriever model carrying the homozygous loss-of-function mutation c.4176insC p.(Phe1393Leufs*1395) in ABCA4 presented with

Table 2. Summary of current animal models for Stargardt disease.

Abca4 knock-out (Abca4−/−, Abca4+/−) mice

	Functional defects		Multimodal retinal imaging				Histopathology			Ref
	Visual impairment	Abnormal ERG	Photoreceptor atrophy	RPE disturbance	Thinning of retina	Increased autofluorescence intensity	RPE lipofuscin accumulation	Photoreceptor degeneration	Others	
Pigmented	-	Delayed dark adaptation	-	-	-	-	Y	-	Elevated PE in outer segments	146
	-	Similar to WT	Similar to WT	Y	Similar to WT	Y	Y	-	-	147
	-	-	-	-	-	Y	Y	Y	-	148
	-	Delayed dark adaptation	-	-	-	-	Y	Y	-	149
Albino	-	-	-	-	-	-	Y	Y	Oxidative stress; complement activation; dead RPE cells with undegraded phagosomes and lysosomal dysfunction; thickness of Bruch's	150–155
Abca4 knock-in mice										
p.(Asn965Ser)	-	-	-	-	-	Y	Y	-	-	156
p.(Leu541Pro) p.(Ala1038Val)	-	Similar to WT and knock-out	-	-	-	Y	Y	-	-	177
Dual knock-out mice										
Abca4−/− /Rdh8−/−	-	Delayed dark adaptation	-	-	-	-	Y	Y	Delayed clearance of all-trans-retinal; atrophy of RPE and Bruch's; drusen deposits; choroidal neovascularization; light worsened retinopathy	157,158
	-	-	-	-	-	-	Y	Y	RPE hypertrophy; shortened outer segments; thickness of Bruch's; oxidative stress	154
Canine model										
c.4176insC (p.Phe1393Leufs*1395)	Y	Delayed dark adaptation	Y	Y	Y	Y	Y	Y	hyperplastic and hypertrophic RPE; thinning of outer nuclear layer	161

RPE; retinal pigment epithelium, WT; wild-type.

a clinical phenotype of photoreceptor degeneration, similar to human STGD1 (161). In addition to the obvious autofluorescent lipofuscin in the RPE that was also observed in mouse models, abnormal rod and cone function as well as histopathological findings, i.e. RPE degeneration and thinning of outer nuclear layer were observed in the affected dog (161). Further studies of this dog model may inform correlations among these observable pathological features, helping to explain the human STGD1 disease phenotype and support development of therapeutics as an ultimate goal.

Current therapeutic modalities for STGD1

The eye has many unique features that make it an attractive therapeutic target compared to other tissues. The optical transparency facilitates accessibility from the exterior and enables precise surgical delivery of therapeutic components to the targeted retinal cell layer. With the advances in retinal imaging techniques, non-invasive examination and functional assessment allow safe and repeated measurements of disease progression and therapeutic effects. Additionally, the relative isolation of the compartment and modified immune privilege make it possible to maintain effective therapeutic drug concentration, minimize systemic immune exposure and reduce potential toxicity to other organs that are not targeted (162). This section will highlight therapeutics for *ABCA4*-STGD1 undergoing clinical trials.

Molecular therapies

A wide range of molecular approaches aimed at modulating secondary pathological pathways to overcome *ABCA4* variant-induced metabolic dysfunction are under intense pre-clinical and clinical investigation. For example, a neuroprotection strategy is to add genes that supply naturally occurring neuroprotective factors that may prolong the lifespan of retinal cells rather than specifically addressing the mutated gene or disease pathogenesis (163). Molecular pharmaceuticals under clinical evaluation for the treatment of *ABCA4*-STGD1 are shown in Supplemental Table 1.

Pharmacotherapy targeting vitamin A metabolism and transport

Overall, current pharmaceutical strategies lead to two approaches: generally inhibiting the retinoid cycle or intervening in the function of related transporters to decrease the formation of toxic bisretinoids. The first strategy utilizes retinoid cycle modulators to target key enzymes of the retinoid cycle and slow down chromophore regeneration, exemplified by Emixustat and isotretinoin; or disrupts the transporters of retinol, such as retinol-binding protein 4 (RBP4) antagonists including N-(4-hydroxyphenyl) retinamide (fenretinide), A1120, BPN-14136, STG-001 and LBS-008 (tinlarebant).

Emixustat hydrochloride (ACU-4429) is an orally administered small molecule that acts by inhibiting the retinoid cycle isomerase RPE65, the crucial enzyme involved in regeneration of chromophores (refer to Figure 6). By suppressing the conversion of all-*trans*-retinyl ester to 11-*cis*-retinol and concomitantly suppressing rod photoreceptor activity through decreased rhodopsin regeneration, Emixustat is thought to reduce accumulation of toxic lipid-retinoid by-products. In an early phase 1a study (164), a dose-dependent suppression effect, manifested as slow recovery in rod-derived b-wave in ERG, was observed in the group treated with Emixustat. Since the suppression effect was reversible and returned to the baseline by day 7, oral administration on a daily basis was recommended. The subsequent phase 1b study (165) exploring the pharmacokinetics, tolerability and safety of Emixustat that was orally administered into 40 healthy individuals revealed that systematic adverse events were minimal, while milder ocular adverse events comprising chromatopsia, followed by vision blur and reduction of visual acuity were reported in 67% of the subjects who received Emixustat doses 20 mg. However, these ocular-related side-effects were reversible and resolved within 7 to 14 days after completion of the study. In January 2017, the application of Emixustat was expanded to the treatment of STGD1, in two trials. The phase 2 clinical trial (NCT03033108) with 23 participants, aiming to study the pharmacodynamics and assess the safety of Emixustat during a one-month time frame has been completed, the outcome of which is yet to be released. Meanwhile, a phase 3 placebo-controlled trial (NCT03772665) has been activated, with the purpose to determine whether Emixustat can rescue the macular atrophy progression in subjects with STGD1.

Several other potential drugs for the treatment of STGD1 are under preclinical evaluation. Isotretinoin, a conventional drug for acne treatment, is one such drug. A side effect during acne treatment is that patients experienced delayed dark adaptation, due to its inhibitory effect on 11-*cis*-retinol dehydrogenase in RPE cells (166). Such a side effect of slowing down rhodopsin regeneration however may reduce levels of A2E molecular precursor, thus reflecting the possible therapeutic effects in the correction of A2E accumulation (167). Although isotretinoin has shown the ability to block A2E formation and lipofuscin accumulation in an $Abca4^{-/-}$ mouse model of STGD1 (166), evidence for efficacy in human is currently lacking. Side effects, such as mild delays in dark adaptation (166) and decreased night vision, observed in those under isotretinoin treatment for acne (168) should be further evaluated before clinical trials in STGD1 patients.

Studies have shown that biosynthesis of bisretinoids depends on the influx of serum retinol (169), and dietary supplementation with retinol increases lipofuscin accumulation in both liver and RPE cells of $Abca4^{-/-}$ mice (152). Accordingly, methods of reducing the circulating retinol are hypothesized to reduce bisretinoid levels. After being secreted from the liver, retinol from the diet binds to specific retinol-binding protein 4 (RBP4) and transthyretin (TTR) and is transported to extrahepatic organs and tissues. The formation of a tertiary complex increases the molecular weight in circulation, therefore avoiding its loss through rapid glomerular filtration. Inhibition of the retinol-induced interaction of RBP4 and transthyretin (TTR) may compromise the uptake of serum retinol to the retina, thereby decreasing the formation of lipofuscin fluorophores (170).

A synthetic retinoic acid analogue, fenretinide, or N-(4-hydroxyphenyl) retinamide (HPR), can efficiently displace retinol from RBP4 under physiological conditions,

thereby accelerating the clearance of retinol in serum. In animal models, administration of fenretinide produced commensurate reduction in retinol level in both serum and retinoid cycle and lowered subsequent lipofuscin accumulation in RPE cells (169). Although a modest delay in dark adaptation was observed in the study (169), other physiological indicators such as light sensitivity of photoreceptors, 11-*cis*-retinal regeneration kinetics, and phototransduction process remained normal, suggesting that fenretinide may not interfere with retinoid cycle rates while reducing the accumulation of lipofuscin fluorophores as a therapeutic effects. However, fenretinide at higher concentrations was observed to induce apoptosis of human retinal pigment epithelial (ARPE-19) cells cultured *in vitro*, through RAR mediated generation of reactive oxygen species, expression of DNA damage-inducible transcription factor 153 and stress response protein (171).

A1120 is another molecule that aims to lower the serum retinol levels. Like fenretinide, A1120 displaces retinol from RBP4, but does not act as an agonist to RAR, sparing patients from RAR-mediated side effects (170). Nicoleta et al. observed that after oral administration of A1120 (30 mg/kg) to $Abca4^{-/-}$ mice for a period of 6 weeks, certain retinoid cycle retinoids were depleted and A2E accumulation decreased in A1120-treated group (170). Unlike other retinoid cycle modulators, the capability of A1120 for reducing lipofuscin bisretinoid production in the retina is not associated with measurable suppression of the retinoid cycle, which shows the favorable safety profile of this non-retinoid RBP4 antagonist (170). In addition to the therapeutic benefits as with A1120, BPN-14136, another non-retinoid RBP4 antagonist was able to normalize the dysregulated complement system in the retina of $Abca4^{-/-}$ mice after 12 weeks of oral administration at a daily basis (172). No trials to date, however, involve these two RBP4 antagonists for the treatment of STGD1. In contrast, STG-001 is another RBP4 inhibitor which is currently in phase 2a trial comparing two doses for 28 days (NCT04489511). Similarly, LBS-008 (tinlarebant), another inhibitor of RBP4 has been given to healthy subjects in a phase 1 trial (ACTRN12618001823268).

Inhibitors of lipofuscin production
Reduction of toxic by-products can also be achieved by intervening in the chemical reactions underlying formation of condensed compounds. By replacing the C20 hydrogen atoms of vitamin A with deuterium, the binding strength of the C20 carbon-hydrogen bond of retinaldehyde-PE, a compound formed between retinaldehyde and PE is strengthened. Since the cleavage of the C20-H bond is the rate-limiting step in synthesis of vitamin A dimers, the deuterated form of vitamin A is therefore less able to form dimers and cytotoxic lipofuscin (173). Ma et al. showed that compared with the control group fed vitamin A at its natural isotopic abundance, $Abca4^{-/-}$ mutant albino mice raised on diets containing C20-D3-vitamin A exhibited reduced A2E levels after three months (155). In addition, decreased levels of lipofuscin granules were observed in $Abca4^{-/-}$ mice after treatment with C20-D3-vitamin A for 6 months, and the function of the retina improved after a year compared with untreated animals (155). Similar therapeutic potential was also observed in another study, which further showed that administration of

C20-D3-vitamin A normalized the dysregulated complement system without impairing retinal function (174). After completing the phase 1 safety study of C20-D3-vitamin A in 40 healthy volunteers (NCT02230228), a phase 2 clinical trial (NCT02402660) involving STGD1 patients was initiated, aiming to determine its long-term safety and tolerability. At the time of submission, no results have been released from these trials.

Neuroprotective strategies
Neurotrophic agents delivered into the eye, particularly into the vitreous or subretinal space, may preserve photoreceptors and RPE cells. The factors include ciliary neurotrophic factor (CNTF), glial cell line-derived neurotrophic factor, brain derived neurotrophic factor, fibroblast and lens-epithelium-derived growth factors, pigment epithelium-derived factor, X-linked inhibitor of apoptosis, erythropoietin and its derivatives, heme oxygenase 1, superoxide dismutase and catalase, synthetic bile acids, progesterone, dopamine-based therapies and others (163,175–179). Among these factors, CNTF has had the fastest trajectory to clinical trials for the treatment of retinal degeneration.

After obtaining safety and efficacy data from animal studies (180), a phase 1 clinical trial (181) and two subsequent phase 2 trials (182) using CNTF expressing cell implants for RP were conducted. While encapsulated cell implants, as a mode of administration have shown promise in terms of delivering viable cells secreting CNTF (181), therapeutic benefits on visual acuity were not observed in RP patients, either short-term (182) or years after the CNTF treatments (183). Considering that the efficacy for treatments that delay photoreceptor degeneration may be questionable, use of neurotrophic agents also lack guidance regarding optimal dosing, timing, route of administration and combinations of neurotrophic agents. In addition, the exogenous properties inevitably raise safety concerns, and all of these issues have limited the further progression of neurotrophic agents in clinical trials.

As complement-associated inflammation contributes to the progression of STGD1 (184), its modulation provides therapeutic potential. Subretinal injection of recombinant adeno-associated virus encoding complement receptor 1-like protein y (CRRY), a significant complement negative regulatory protein of mouse that prevents host cells from complement attacks by impeding the formation of the cytolytic membrane attack complex, has been shown to increase visual chromophore levels while reducing the level of bisretinoid in a STGD1 mouse model (185). More importantly, photoreceptor degeneration was delayed in the treated mice. This result not only implicates inappropriate complement activation in the pathogenesis of STGD1 but also presents an alternative treatment for STGD1 and other retinal diseases associated with dysregulated complement signaling. The complement factor C5 inhibitor (Zimura) has recently entered clinical trials (NCT03364153) for safety and efficacy evaluation in STGD1 patients (186). The primary outcome presented by the mean rate of change in the area of ellipsoid zone defect measured by en face SD-OCT within a time frame of 18 months was submitted, and the results are highly anticipated.

Saffron also works as a neuroprotectant. The constituents, crocetin and crocin, have been suggested to counteract retinal oxidative damage and protect retinal cells from apoptosis (187). In a clinical trial (NCT01278277) of oral saffron versus placebo for six months, saffron was well tolerated among the 31 recruited patients with *ABCA4*-STGD1, but no measurable improvement was recorded, warranting a long-term study to evaluate therapeutic effects on the progression of retinal dystrophy in STGD1 (187).

Preclinical studies aiming to find a treatment for STGD1 are emerging, including retinylamine and its derivatives (188,189), G protein-coupled receptor modulators (190) and dietary supplements (191), and have been reviewed by other authors (192,193).

ABCA4 gene- or mutation-specific therapies

With the broad aim of introducing therapeutic nucleic acids into targeted cells, gene therapy approaches can be classified into (1) gene replacement that introduces a functional copy of the causative gene into affected cells, without addressing the mutation itself and is generally applicable for diseases caused by loss-of-function mutations (194; 2) gene editing utilizes highly specific nucleases to repair the pathogenic mutation in the endogenous affected allele, with the aim of restoring the wild-type DNA sequence and functional protein expression (195; 3) splice modulation that induces alternatively spliced transcripts via administration of antisense oligonucleotides or specialized small molecules. By targeting selected splicing motifs as needed, a disrupted reading frame due to mutations can be restored, or exons carrying disease-causing mutations can be removed during pre-mRNA splicing process, with the aim to generate functional protein (162).

Gene replacement therapies

There are presently two major vector platforms for gene replacement therapy, viral and non-viral. A number of viral vectors have demonstrated tropism for specific ocular cells in tissue culture and animal models (196), among which, adenovirus, adeno-associated virus and lentivirus are the most common vectors used to deliver therapeutic cargoes. Unlike viral systems, non-viral delivery approaches involve administration of naked DNA, or in combination with chemical substances such as cationic lipids, peptides, polymers and nanoparticles, or physiochemical methods such as electroporation, iontophoresis and microinjection (197).

The minimal functional size of *ABCA4* cDNA is 6.8 kb and hence exceeds the cargo capacity of recombinant adeno-associated virus (rAAV, ≤ 4.7 kilobases). For years, efforts have focused on artificially expanding AAV packaging capacity. In 2008, a landmark publication reported that expression of murine Abca4 protein was localized to rod outer segments following transduction with recombinant AAV2/5 packaging a large expression cassette containing single-stranded *Abca4* (8.9 kilobases) (198). Furthermore, subretinal delivery of the vector in the *Abca4*$^{-/-}$ mouse model resulted in significant reduction of lipofuscin granules (mainly fluorophore A2E) accumulated in RPE, and the ability of photoreceptors to recover from light desensitization was significantly improved (198).

Although three independent research groups later noticed the discrepancy between heterogeneously partially packaged sequences (no larger than 5.2 kilobases) and full gene expression cassettes (> 5 kilobases) (199–202), the ability of *ABCA4* gene fragments to recombine once inside the target cells later inspired dual AAV vector gene replacement approaches. In this way, *ABCA4* gene fragments are assembled through inverted terminal repeat-mediated concatemerization (the trans-splicing approach) (203), homologous overlapping sequence-mediated extension (the overlapping approach) (204), or a combination of the two (the dual hybrid AAV approach) (205). Trapani and colleagues have compared the efficiency of dual AAV strategies and AAV oversize vectors to deliver *EGFP, ABCA4* and *MYO7A in vitro*, as well as in the retina of mouse and pig (206). In their study, dual AAV strategies outperform AAV oversize vectors in terms of transduction levels both *in vitro* and *in vivo*. Dual AAV trans-splicing vectors and AAV hybrid vectors containing alkaline phosphatase recombinogenic sequence efficiently transduced both photoreceptors and RPE cells, albeit less efficiently compared to the single normal-sized AAV vectors. However, only dual AAV overlapping vectors efficiently transduced mouse RPE cells rather than photoreceptors. Notably, subretinal delivery of dual AAV trans-splicing and hybrid vectors improves the retinal defects of the *Abca4*$^{-/-}$ mouse model, with neither electroretinography (ERG) nor retinal histological abnormalities detected during 3–8 months follow-up post-treatment. Other studies also reported efficient transduction using dual AAV vectors in photoreceptors of *Abca4*$^{-/-}$ mouse and pigs (207–209), and methods to improve transduction efficiency of vectors delivering *ABCA4* have been discussed elsewhere (208,210).

Lentiviral vectors have gained attention for the delivery of large genes such as *ABCA4* due to its ~8 kb cargo capacity. Kong et al. showed that delivery of wild-type *ABCA4* cDNA via subretinal injection of equine infectious anemia virus (EIAV)-derived lentiviral vectors into newborn *Abca4*$^{-/-}$ mice reduced A2E accumulation (211). Although the transduction efficiency of photoreceptors in the injected area was low (from 5%-20%), ERG evaluation indicated that the treated group benefited from subretinal injection of the *ABCA4* lentiviral vectors, suggesting that this level of photoreceptor transduction was sufficient to rescue phenotypes in the *Abca4*$^{-/-}$ mouse model (211). Another preclinical safety study demonstrated that a single injection of EIVA-derived lentiviral vector is safe and well-tolerated in rabbits and macaques, though a slight and transient cellular inflammatory response was observed in the treated eyes (212). A phase 1/2 clinical trial (NCT01367444) employing EIAV-derived lentivirus pseudo-typed using vesicular stomatitis virus glycoprotein (drug SAR422459) for subretinal delivery of *ABCA4* cDNA was been terminated early this year, and no efficacy data was published. Its phase 1/2 follow-up trial (NCT01736592) aiming to evaluate the long-term tolerability of SAR422459 in patients with STGD1 is activate, but yet to recruit patients (Supplemental Table 2). Non-viral delivery of exogenous nucleic acids, either naked or conjugated with chemical carriers, is an alternative approach that theoretically should allow the delivery of much larger

nucleotide fragments without triggering severe immune responses, compared to viral administration. Han et al. delivered a DNA construct compacted with polyethylene glycol-substituted 30-mer lysine peptides (CK30-PEG) carrying the human *ABCA4* cDNA (6.8 kilobases) to the subretinal region in eyes of *Abca4*$^{-/-}$ mice (213,214). Transgene expression was persistent for up to 8 months, and both functional (delayed dark adaptation indicated by ERG) and structural (fundus lipofuscin accumulation) phenotypes of STGD1 were significantly improved (213,214). This study provided the first evidence of non-viral delivery of human *ABCA4* to photoreceptors.

Beneficial features of the compacted nanoparticles have been discussed in various studies, including superior tolerance even after repeated injection (215), persistent gene expression (216), the absence of adverse events such as ocular/systemic toxicity and insertional mutagenesis (217,218), and absence of significant local inflammatory responses or toxicity (219).

Gene editing

In contrast to gene replacement therapy, gene editing targets the mutant gene in order to restore the wild-type sequence. Gene editing technologies exploit programmable nucleases including Meganucleases (220), transcription activator-like effector nucleases (TALEN) (221), zinc finger nucleases (ZFN) (222) or clustered regularly interspaced short palindromic repeats (CRISPR)–associated nuclease Cas9 (223). The recent developments of CRISPR-Cas9 have revolutionized various fields of biotechnology and biomedicine. Since first utilized in the early-1990s (224), the highly specific endonuclease platform has been developed to enable precise genome editing by introducing DNA double stand breaks (DSBs), single-strand breaks (nicks) (225) or base editing (226).

Overall, the development of gene editing for retinal disease splits in two directions. In the *ex vivo* approach, gene editing is performed in patient-derived cells to correct mutations, followed by reprogramming cells into iPSCs and differentiating to RPE or photoreceptors for subsequent transplantation. In contrast, the *in vivo* approach is to target mutations in retinal cells *in situ* (195,227–231) through delivery of editing machinery and repair templates using the gene delivery vectors discussed above. Once all elements, such as endonucleases, the template of the gene of interest and various modifying agents, are delivered to the target cells via intravitreal or subretinal injection, DSBs can be induced at the targeted genomic locations by endonucleases. Subsequently, endogenous host proteins detect and repair the DSB through one of the three different pathways: the error prone non-homologous end-joining (NHEJ) pathway, or with high fidelity through homology directed repair (HDR), or microhomology mediated end-joining pathway (MMEJ) (195). NHEJ, during the whole cell cycle, repairs the lesion by directly reconnecting the two DSB ends in a process that does not require templates, whereas HDR requires an exogenous DNA template with sequence homology to the lesion site to integrate into the DSB during repair in the S- and G2- phase of the cell cycle (232). Although NHEJ-mediated DSB repair can be rapid and accurate, it can result in small deletions, insertions or substitutions that may cause frameshifts, leading to mRNA degradation or production of

non-functional truncated proteins (227). MMEJ, as an alternative pathway to the classic NHEJ, aligns the micro-homologous sequence with the DSB ends for repairing small deletions, insertions and chromosome translocations (233). Komor et al. engineered fusions of CRISPER/Cas9 and a cytidine deaminase that can mediate the direct conversion of cytidine to uridine without cleavage of double-stranded DNA (226). This "base editor" converts cytidines within a window of approximately five nucleotides and has shown editing efficiencies of 15–75% with typically ≤ 1% insertion/deletion formation in mammalian cell lines. Soon afterwards, a new class of adenine base editors (ABEs) was developed to convert A or T to G or C in DNA, with approximately 50% efficiency and ≤ 1% incidence of insertion/deletion in human cells (234).

Gene editing is contingent upon the safe and efficient viral- or non-viral delivery of DNA, RNA or protein to the target of interest. Before processing to clinical development as a treatment for *ABCA4*-related retinopathy, gene editing faces several hurdles including inefficient delivery of the expression cassette, and integration of endonucleases and the repair template. The potential adverse and off-target effects must also be considered. Of note, recent studies point out human pluripotent stem cells with a functional *TP53* gene (encoding P53) have severely reduced efficiency of precise genome editing (235), and such efficiency can be improved by p53 inhibition (235,236). Given that P53 inhibition may expose targeted cells to severe adverse events, such as chromosomal rearrangement, off-target mutations and tumorigenic mutations, both risks and benefits must be cautiously evaluated when developing CRISPR-Cas9-mediated therapies (235,236).

Splice modulation methods

Antisense oligonucleotide (AONs) mediated splice modulation is gaining increasing attention as a strategy to overcome several specific disease-causing mutations. AONs are short (8–50 nucleotide) single-stranded nucleic acids or nucleic acid analogues, complementary to the target sequence, according to the Watson–Crick base pairing principle (162). The use of AONs has been expanded to invoke various mechanisms to modify gene expression at the mRNA level, including 1) induce exon skipping to bypass nonsense mutations and mutations that cause reading frame shift; 2) block cryptic splice sites that are abnormally activated; 3) impede splice silencers located near exons in such a way as to enhance exon recognition (237).

Gérard et al. have provided *in vivo* evidence generated from mouse that intravitreal injection of 10 nanomoles of *Abca4*-specific AONs targeting the exonic splice enhancer near exon 10 resulted in a shortened transcript, lacking a 104 bp sequence that partially overlaps exon 9 and 10 (238). The splicing-modulation effect was maintained for 10 days after treatment (238). Albert and Garanto et al. reported that in STGD1 patient-derived photoreceptor progenitor cells, AON administration can prevent inclusion of the aberrantly activated 345-nucleotide pseudo-exon caused by two neighboring deep-intronic *ABCA4* mutations (c.4539+2001G>A and c.4539+2028C>T) (129,130). Other *in vitro* splice intervention studies also demonstrated the ability of AONs to eliminate pseudo-exons caused by intronic *ABCA4* mutations (132,135).

A clinical trial employing AONs to target the deep-intronic mutation of *CEP290* was initiated (NCT03140969). Recently published outcomes showed vision improvement without adverse events after intravitreal injection of AONs (239). As increasing numbers of splice-affecting variants in *ABCA4* are being revealed, AON intervention is anticipated to yield additional promising therapeutic outcomes by addressing aberrant splicing events. However, the drawback of AON strategies is the lack of one-drug-fits-all approach and individualized AON design will be required for many mutations, therefore limiting the broad application of AONs-mediated therapy to patients with uncommon mutations (131). On the other hand, AONs are offering new hope to patients with rare diseases, with the recent FDA approval of "Milasen" for the treatment of a rare form of Batten's disease, occurring within record breaking time (a year from the laboratory to get to the patients) (240), highlighting AON intervention as potential precision medicine for inherited retinal diseases.

Cell replacement, neuromodulation and bionic vision

Retinal pigment epithelium and photoreceptor transplantation

Due to the extreme lack of donor cell resources and post-transplant safety issues, the development of RPE and photoreceptor cells transplantation as therapeutics has been limited. Pluripotent stem cells developed in recent decades hold great potential for the treatment of retinal degenerative diseases. Current promising cell transplantation strategies that underwent clinical trials relied on two donor cell sources, human embryonic stem cells (hESCs) and somatic cell reprogrammed induced pluripotent stem cells (iPSCs) (Supplemental Table 3).

For effective RPE cell replacement therapy, key features of RPE in terms of gene and protein expression profiles as well as morphological, behavioral and physiological capabilities must be achieved. Both hESC- and iPSC-derived RPE cells are well suited for large-scale production of human RPE cells that display the morphological and functional similarities of primary RPE cells. These cells grow as monolayers comprised hexagonal, pigmented cells with apical microvilli (241–243), present correct apical-basal polarity with secretion of growth factors (244–246) and are capable of phagocytosis of outer segments (242,247–249). The gene expression profiles of ESC- and iPSC-derived RPE cells are similar to adult primary RPE cells (248,250), though ESC-derived RPE cells show variability in the expression of adhesion junction and membrane transport genes (251). Variability between human ECS- and iPSC-derived RPE is also seen in the genomic DNA methylation patterns, which is dynamically regulated during RPE differentiation (252), and in addition, iPSC-derived RPE cells may retain the epigenetic markers associated with the cell types they were originally derived from (253).

The properties and functions of transplanted ESC- and iPSC-derived RPE cell have been investigated in animal models. Transplanted cells retain morphological and functional features similar to native RPE-like tissues *in vivo*, such as "cobblestone" morphology with pigmentation and the ability of phagocytosis capability (254–257). Maintenance of photoreceptors and visual function has been observed in rat models, in which stem cell-derived RPE cells were transplanted under the retina (257,258).

Translational application of human ESCs-derived cells is now being transitioned to phase 1/2 clinical trials (Supplemental Table 3). The first priority of these clinical trials concern safety, including assessments of serial vital signs and adverse events; monitoring of transplantation tolerance, integrity and rejection; local and systemic infection; and tumorigenic transformation. In 2005, the preliminary results of a clinical study (NCT01345006) aiming to evaluate safety and tolerability of subretinal injection of human ESCs-derived RPE in patients with STGD1 were released (259). During a median follow-up observation period of 22 months (up to 37 months), the data showed increased and stable best-corrected visual acuity as well as improved vision-related quality-of-life measures (260). No signs of hyperproliferation, tumorigenicity, ectopic tissue formation, transplant rejection or inflammation were observed. A phase 1/2 trial (NCT01469832) involving 12 patients with advanced STGD1 attempted to study the retinal structure and function in the treated region after subretinal transplantation of human ESC-derived RPE cells. No obvious benefit was observed in best-corrected visual acuity and microperimetry, and hyperpigmentation of focal areas and localized retinal thinning and sensitivity reduction may suggest potential harm arising from the treatment (261). Although the evidence to date suggested many outstanding merits, the ethical controversies surrounding the use of hESC have hampered their widespread investigation. Moreover, despite hESC and its differentiated derivatives being less susceptible to immune rejection (262), patients who received the allograft transplantation, differentiated from stem cells, may also need to take lifelong immunosuppressive therapies.

The loss of photoreceptors is one of the pathological features of STGD1. Therefore, replacement of these cells by transplantation could offer a potential treatment. For effective photoreceptor transplantation, a precise host synaptic connection with the transplanted photoreceptors must be established (263), in order to restore the light sensitive function of the recipient retina. Among various cell transplantation approaches that have been investigated during the last three decades, transplantation of photoreceptor precursor cells is most promising.

Precursor cells have been obtained from postnatal mouse retina or isolated from retinal organoids derived from hESC and iPSC and transplanted in retinal degeneration animal models (264). Precursor cells sourced from early postnatal mouse were capable of integrating into the outer nuclear layer of adult retina, forming synaptic connections, and differentiating to acquire specialized features possessed by mature rods as well as restoring some visual function (265). Integration of up to 26,000 rods taken from postnatal Nrl-GFP donor mice (266) were distributed across more than 50% of the retina in the $Gnat1^{-/-}$ mouse model (267), and light responses of transplanted photoreceptors were also recorded (267). The improvement of some other visual activities after transplantation of photoreceptor precursor cells was reported in other studies (268–270). These preclinical outcomes indicate the feasibility of photoreceptor as a potential treatment for retinal dystrophies.

The technology of *in vitro* expandable pluripotent stem cells would further facilitate scalable generation of specific cell types, including RPE cells, photoreceptors and 3D retinal organoids. Protocols for derivation of specific retinal cells and retinal organoids from pluripotent stem cells have been reviewed elsewhere (264,271). Efforts in recent decades have led to the accumulation of new knowledge on pluripotent stem cells, bringing hope for the application of these cells in clinical development.

Cell-based preservation therapy

Numerous types of cells have been investigated for their capacity to promote the survival of retinal cells through their paracrine trophic effect, including mesenchymal stem cells, vascular precursor cells, adipose stromal cells and neural stem/progenitor cells derived from various sources (241,272,273). Nonetheless, only few of these strategies have been clinically tested on patients with retinopathy or ophthalmic syndromes.

CD34+ haematopoietic stem cells have been explored as therapeutics in animal models of retinal ischemia and degeneration. A landmark observation unveiled that systemic circulation makes these cells to be recruited in response to tissue injury, thus facilitating tissue regeneration and angiogenesis (274). Preclinical data indicate that the preservative therapeutic effects of CD34+ cells also depend on their paracrine trophic effects, by secreting neurotrophic and proangiogenic factors (reviewed by Susanna et al (275)). A phase 1 trial using autologous bone marrow-derived CD34+ cells is currently being tested as a preservation treatment involving STGD1 patients (NCT01736059). Although the number of participants of this trial being extremely limited (only 2 STGD1 patients), preliminary results indicated that the intravitreal injection of autologous bone marrow-derived CD34+ cells was well tolerated by the patients. Over 18 months, no ocular or systemic adverse effects or hyperproliferation were observed, suggesting the safety and feasibility of this approach (276). However, a recent report highlighted the risk of adipose stem cells implantation (277). In this report, three patients with AMD experienced severe bilateral visual loss, associated with serious complications including ocular hypertension, haemorrhagic retinopathy, vitreous haemorrhage, combined traction, rhegmatogenous retinal detachment, or lens dislocation, after they received intravitreal injection of autologous adipose tissue-derived "stem cells." The visual acuity of the three patients ranged from 20/200 to no light perception after one year of transplantation.

It is worth mentioning that an appreciable number of functional photoreceptors are necessary for both cell-based replacement and preservation therapies. Either transplanting RPE cells too early (host RPE is still predominant) or too late (host RPE has gone) may lead to potential complications (241). It has also been suggested that genotypes of patients, as well as retinal structural evidence measured by optical coherence tomography and en face autofluorescence, should be considered as the inclusion criteria in cell transplantation trials (278).

Optogenetics

For patients with nearly complete loss of photoreceptors, there is very little chance of benefit from other treatments such as neuroprotective strategies and mutation-specific therapies. In such case, optogenetics can provide an alternative therapeutic strategy (279). Optogenetics is generally based on the idea that, by genetically introducing light-sensitive protein (mainly opsin-based protein) into cell membrane, those inner retinal neurons that are intrinsically light insensitive and temporarily exempted from the primary damage in retinal diseases can be artificially photosensitized. The optogenetics toolkit at its present stage comprises two distinct families: animal opsin and microbial opsin. Lin et al. reported that expression of the light-sensitive protein melanopsin in retinal ganglion cells, a type of retinal neuron that is intrinsically photosensitive yet contribute little to vision, can enhance visual function regarding behavioral light avoidance response and discrimination of light stimulus in *rd1* mice (280). Later, Cehajic-kapetanovic et al. ectopically expressed human opsin in the *rd1* mouse model and effectively restored the visual responses induced by simple light pulses, luminance increases and naturalistic movies, and behavioral responses to light pulses at the intensity levels as the natural indoor environment (281). Though the reliability of these promising results obtained in animal models await further evaluation in humans, vision restoration through light stimulation including optogenetics is rapidly evolving from an idea into reality (282).

Microbial opsin, compared to animal opsins, owns less complexity and requires a lesser collaborative neuron network for photoresponses. Microbial opsins are usually light-activated ion channels or pumps, such as channelrhodopsin, halorhodopsin, bacteriorhodopsin and proton pumps (comprehensively reviewed in (282)). Based on the very first report showing that ectopic expression of channelrhodopsin-2 in the residual inner retinal neurons in photoreceptor-deficient *rd1* mice facilitated the restoration of vision (283), further advancement of optogenetics has aimed at inducing specific expression of opsins in selected target cell types. Busskamp et al. reported that expression of archaebacterial halorhodopsin can replace the native phototransduction cascade and reactivate light-insensitive cones in mouse model of RP (284). The resensitized photoreceptors were able to activate retinal cone pathways and drive the sophisticated secondary retinal circuit functions (284). By using human *ex vivo* retina, experiments further showed that defective photoreceptors can regain light sensitivity with the expression of halorhodopsin (284). It is also suggested that patients with cones, light-insensitive but with cell bodies remaining in the central area, can be considered as candidates for the corresponding therapy (284).

Varying degrees of visual improvement were also observed in other studies that performed optogenetic stimulations of inner retinal neurons in animal models of retinal dystrophy (285,286). At present, the translation of optogenetics combined with gene therapy to the clinical realm is in progress for RP (NCT02556736 and NCT03326336). Last year, Garita-Hernandez reported that after transplanting optogenetically transformed photoreceptor precursor cells, partial visual function was restored in blind mice (287). Additionally, the transplantation of cones differentiated from human iPSCs, expressing the chloride pump, contributed to light-driven responses in both photoreceptors and ganglion cells in blind mice, indicating that by integration of stem cell technology and

optogenetics, structure and function of retina can be restored (287).

Artificial retinal prosthesis

Similar to optogenetics, retinal prosthesis is another strategy that stimulates inner retinal neurons that remain functional in cases with demise of photoreceptors and RPE cells, thereby enabling the signals to be transmitted along the intact visual pathway to the corresponding cortex for processing. The difference is that retinal prostheses work by electrical stimulation. According to the location of the implants, retinal prosthetics can be categorized into epiretinal prosthetics, subretinal prosthetics and suprachoroidal prosthetics. The advances of each prostheses in the related retinal research, with particular emphasis on the engineering and clinical specifics has been covered elsewhere (288). Another review discussed the principles and challenges of each bioelectrical approach (282).

At present, the most successful device is the Argus II Retinal Prosthesis System. With the approval from Conformité Européenne in 2011 and the U.S. Food and Drug Administration in 2013, worldwide commercial implantation of Argus II has commenced in patients with retinal diseases, including RP and to a lesser extent, AMD and choroideremia (288). The preclinical animal and human studies that led to the development of Argus II and the outcomes of these studies in terms of safety profile and function improvement have been comprehensively reviewed (289). Recently, Endo et al. reported that after implantation with a suprachoroidal-transretinal stimulation (STS) retinal prosthesis in the right eye of a patient with advanced STGD1 with residual vision, the performance of a targeted reaching movement was consistently improved during one-year follow-up period (290). This study brings hope for integrating retinal prosthesis with the treatment for STGD1.

Conclusion remarks and future directions

STGD1 is currently incurable, and once diagnosed, is progressive and irreversible. The large variability in phenotype and disease progression rates even among family members carrying the same mutations remain a major obstacle for clinical trial design and patient selection for clinical trials. The therapies currently applied towards the treatment of STGD1 attempt to delay or halt vision loss in patients, but no treatment has been proven to restore vision. The search for effective and safe therapeutic options has led to the development of two branches of studies aiming to model human STGD1. One is the development of animal models, and the other one is to isolate the appropriate cell lines of interest. The currently available animal models cannot fully mimic human STGD1 (291). Thus far, some of the potential therapeutic strategies such as gene therapy, cell-based therapeutic platforms and pharmaceuticals as discussed in this review have shown great promise, though various hurdles remain to be addressed in order to achieve safe and effective therapies. In this context, the uncertainties such as the impact of high mutational heterogeneity of ABCA4 for

certain therapies, and difficulties including the lack of consensus in selecting the optimal treatment window, administration route, sites and dosages; the lack of a stronger evidence for the molecular mechanisms underlying ABCA4-associated STGD1, based on animal models or cell lines; the lack of sufficient clinical evidence supporting therapeutic longevity, safety and efficacy; and the lack of long term observation of side effects or potential deleterious events during treatment etc., have hindered progress and require ongoing efforts of generations of researchers.

To conclude, it is important to reflect on several key points. Firstly, the cautious pathogenicity assessment of ever-increasing numbers of ABCA4 variants and a better understanding of their molecular consequences is essential for developing correlations between genotype and phenotype, as well as exploring mutation mechanism-dependent treatment strategies, regardless of whether they are targeting the cause or the consequences of STGD1. In addition, the combination of current therapeutic options for STGD1 with the current advanced ophthalmological surgery techniques could be the key to increasing the success in maintaining, or ideally restoring visions in patients with STGD1. Last but not least, in order to achieve optimal maintenance or improvement of vision, attempts to develop neuroprotective treatments for STGD1 using mutation-dependent and/or -independent strategies should continue to be investigated. In addition to their therapeutic effects, the safety profiles of these treatments for various phenotypes of STGD1 warrant careful consideration, given the large number of mutations and the varied impact of these on ABCA4 splicing and ABCA4 protein function.

Disclosure Statement

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