**Title:**

Retinol dehydrogenase 12 (RDH12): Role in vision, retinal disease and future perspectives

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## Highlights

- Retinol dehydrogenase 12 (RDH12) structure, function and role in vision.
- Disease mechanisms and clinical phenotype of *RDH12* retinopathies.
- Therapeutic avenues and consideration for future research.


## Abstract

Retinol dehydrogenase 12 (RDH12) is an NADPH-dependent retinal reductase, which is expressed in the inner segments of the photoreceptors. It functions as part of the visual cycle, which is a series of enzymatic reactions required for the regeneration of the visual pigment, and has also been implicated in detoxification of lipid peroxidation products. Mutations in *RDH12* have been linked to Leber congenital amaurosis (LCA) and autosomal dominant retinitis pigmentosa. A number of *in-vitro* studies have shown that mutations in *RDH12* result in little or no enzyme activity. Knockout mouse models however do not recapitulate the severe phenotype observed in patients, resulting in a limited understanding of the disease mechanisms. With gene replacement and small molecule drugs emerging for inherited retinal dystrophies, herein we provide a review of RDH12 structure, its role in vision and the current understanding of disease mechanisms linked to clinical phenotype to support therapeutic development.



## Keywords

Retinol dehydrogenase 12 (RDH12); visual cycle; Leber congenital amaurosis (LCA).

## 1. Introduction

Retinol dehydrogenase 12 (RDH12) is an NADPH-dependent retinal reductase that functions as part of the visual cycle; involving a series of enzymatic reactions that regenerates the visual pigment, 11-cis retinal. *RDH12* has 7 coding exons, is located on chromosome 14q24.1 and encodes a 316 amino acid protein, with an estimated molecular weight of 35 kDa. It is expressed in the inner segments of the photoreceptors (Belyaeva et al., 2005) and according to the human protein atlas database, is also expressed in the skin, kidney and liver. Mutations in *RDH12* are primarily associated with Leber congenital amaurosis (LCA) type 13, an early onset retinal dystrophy, presenting in early childhood and accounting for approximately 10% of all LCA cases. One case of a heterozygous variant has also been implicated in autosomal dominant retinitis pigmentosa (RP) (Fingert et al., 2008). This review will focus on the structure of RDH12, its role in vision, the disease mechanisms, and potential therapeutic avenues.

## 2. *RDH12* retinopathies

Autosomal recessive biallelic mutations in the *RDH12* gene were first identified in three consanguineous Austrian families, with 15 members affected with severe retinal dystrophy (Janecke et al., 2004). A genealogical link was not found between the families, but due to the geographical proximity of the families, it is thought that they were related. The homozygous missense c.677G>A; p.(Y226C) variant was found in all affected individuals. This variant was also found in two non-related Austrian individuals. In COS-7 cells transfected with a vector encoding the *RDH12* p.(Y226C) variant, no enzyme activity was found in the forward or reverse reaction, suggesting a loss of function. A number of *RDH12* autosomal recessive mutations linked to LCA have since been identified (Avila-Fernandez et al., 2010; Benayoun et al., 2009; Coppieters et al., 2014; Mackay et al., 2011; Perrault et al., 2004; Sodi et al., 2010; Sun et al., 2007; Thompson et al., 2005). *RDH12* mutations account for approximately 10% of all LCA cases (Kumaran et al.,

2017). According to the Human Gene Mutation Database, 80 *RDH12* mutations have been reported, 51 of which are missense and 12 are nonsense mutations (HGMD public database accessed April 2019). As shown in Figure 1, mutations span the entire gene, including the conserved regions, with no specific hotspots.

One case of an autosomal dominant *RDH12* variant has also been reported; a heterozygous single base pair deletion c.776delG; p.(E260Rfs*18) resulting in a frameshift and premature termination at codon 277, in 19 affected members of a large 6 generation family (Fingert et al., 2008). Interestingly, compared to the autosomal recessive LCA patients, these patients displayed late onset, relatively mild retinitis pigmentosa.

## 3. Clinical phenotype

Patients with autosomal recessive *RDH12* retinopathy usually present in infancy with early onset visual loss. This is a progressive disease characterised by variable pigmentary retinopathy with peripapillary sparing, RPE atrophy and pronounced central macular changes including pigmentary maculopathy, yellow macular deposits and macular excavation, leading to severe visual impairment and blindness in adulthood (Figure 2). (Aleman et al., 2018; Garg et al., 2017; Li et al., 2017; Mackay et al., 2011; Valverde et al., 2009; Zou et al., 2018).

Aleman et al. (2018) studied 21 paediatric patients from 14 families (age range 2-17 years), most children presented within the first 2 years of life with early onset visual loss of variable levels and displaying inter-ocular asymmetry. Baseline full-field ERGs were undetectable or severely reduced and fundus examination revealed waxy optic disc pallor, vascular attenuation and mid-peripheral pigmentary changes in all patients with variable distribution from localised paravascular to bone spicule macula pigmentation. The macula was affected in all patients ranging from central depigmentation, a denser yellow hue at the

foveal centre, parafoveal bullseye area of depigmentation to foveal chorioretinal atrophy with pigmentation, and in some cases a pseudo-colobomatous configuration. Corresponding fundus autofluorescence (FAF) revealed central hypoautofluorescence, surrounded by hyperautofluorescent lesions which colocalised with bone spicules and hyperpigmentation. There was preservation of the FAF signal in the peripapillary region as per previous observations. SD-OCT shows abnormal macular structure with an almost undetectable outer nuclear layer (ONL) within a thinned foveal centre. This ONL has a normal appearance in the peripapillary retina. The ellipsoid zone (EZ), interdigitation zone (IZ), and external limiting membrane (ELM) bands are not clearly visible for most of the scans. Using multimodal imaging, visual psychophysics, and dark-adapted chromatic pupillometry, severe central cone and rod dysfunction correlated with the central structural abnormalities with milder extra-macular rod dysfunction.

In a recent retrospective chart review of 57 patients by Fahim and colleagues, the average age of onset was 4 years old. Atrophic changes at the macular was a universal finding from as early as age 2, and electrophysiology was markedly reduced in both scotopic and photopic responses. SD-OCT revealed severe structural disorganisation largely after 10 years of age, and 18 patients had peripapillary sparing (Fahim et al., 2019).

A recent cross-sectional report of 38 patients from 38 unrelated families of Chinese descent, age range 3-53 years (median 20 years), with molecularly confirmed homozygous or compound heterozygous *RDH12* mutations had varying diagnoses of LCA, early onset severe retinal dystrophy (EOSRD), autosomal recessive retinitis pigmentosa (ARRP), or cone-rod dystrophy (CORD) (Zou et al., 2018). The most common variant was p.(V146D), followed by p.(R62*) and p.(T49M), accounting for 50% of the cases. Visual acuity varied considerably ranging from no light perception to 20/40 (median was 20/200), but once adjusted

for age, there was no difference in best corrected visual acuity between patients with EOSRD, ARRP, and CORD, although LCA patients had significantly worse vision. Over 55% of patients reported nyctalopia. Variable pigmentation was noted ranging from no pigment with minimal scattered bone spicules to confluent pigment proliferation. They characterised the phenotype into 4 types: (1) Macular coloboma (mostly petal-like) with dense bone spicule pigmentation in the mid-peripheral retina (48.7%); (2) Macular discoloration and widespread bone spicule and/or salt–pepper pigmentation (27.6%); (3) Heavy and confluent pigment proliferation in the mid-peripheral region involving the macular region (18.4%); and (4) Retinal posterior pole atrophy with a relatively normal peripheral retina (2 patients). Overall, the clinical phenotypes were variable, and as yet the determinant factors are not fully understood.

One six-generation family with 19 affected members was found to have autosomal dominant *RDH12* retinopathy (Fingert et al., 2008). They showed a late onset (average age at diagnosis was 28.5 years) retinitis pigmentosa phenotype with intraretinal bone spicule pigmentation and arteriolar attenuation. Some affected individuals maintained good central visual acuity (20/25) until their eighth decade of life. No further cases have been published in the literature.

Most of the studies are cross-sectional observational studies. A prospective deep phenotyping natural history study is required to support future clinical trial development by identifying reliable therapeutic outcome measures. As the phenotype in recessive disease begins so early in life, paediatric age-appropriate metrics are required for accurate data capture, especially in terms of visual function. Applying adult tests may prove inaccurate due to factors such as cognitive skills that are still developing such as working memory and rule comprehension, in addition the tests are typically unsuitable for patients with low vision or poor fixation.

## 4. Structure

Retinol dehydrogenases (RDHs) are members of the short chain dehydrogenases/reductases (SDR) family of enzymes. The SDRs are typically 250-350 amino acids in length and have a relatively low sequence similarity of about 15-30%. However, common to all SDRs is the highly conserved Rossman fold, which is composed of a central β-sheet flanked by 3-4 α-helices, forming the cofactor binding site. The SDRs have two conserved domains: the cofactor binding site (GXXXGXG) and the catalytic site (YXXXK) (Liden et al., 2003). A number of RDHs are involved in the visual cycle, and vary in substrate and coenzyme specificity. RDH8 and retSDR1 are found in the photoreceptor outer segments; RDH12 and RDH11 are found in the inner segments. RDH5, RDH11 and RDH10 are expressed in the retinal pigment epithelium (RPE) (Parker and Crouch, 2010). RDH12 is most closely related to RDH11, sharing 79% sequence similarity (Haeseleer et al., 2002). In RDH12, the cofactor binding site is located at positions 46-52 and the catalytic site at positions 200-204 (Figure 3). Due to the difficulty in expression and purification of RDHs, the crystal structure of RDH12, or that of any other vertebrate RDH, has not yet been solved. A homology model of RDH12, built using the Phyre2 software (Kelley et al., 2015) is shown in figure 3. Hofmann et al. (2016) reported the crystal structure of *Drosophila melanogaster* photoreceptor retinol dehydrogenase (PDH), an orthologue of RDH12, and found it in a dimeric state, thought to be the native conformation, as monomeric PDH in solution was not active. Based on the homology of RDH12 to PDH, it is possible that RDH12 may also function as a dimer.

## 5. RDH12 substrate specificities

Purified RDH12 displays a ~2000 fold higher affinity for NADP+ and NADPH than for NAD+ and NADH, and has a greater affinity for retinaldehydes than retinols. RDH12 functions as a retinal reductase, with highest activity towards all-trans retinal, followed by 11-cis retinal (Figure 4). However, it is unlikely that 11-cis

retinal is metabolised by RDH12 *in vivo*, as according to the visual cycle, 11-cis retinal that enters the photoreceptors is likely to be sequestered by opsins. Binding of CRBP1 to all-trans retinol prevents its oxidation by RDH12 (Belyaeva et al., 2005).

RDH12 can also act on medium chain aldehydes, produced from lipid peroxidation of unsaturated fatty acids (Belyaeva et al., 2005). RDH12 was shown to metabolise the lipid derived medium chain aldehyde nonanal, and inhibit the reduction of all-trans retinal in RDH12 transfected HEK-293 cells, indicating that RDH12 can protect cells from nonanal induced toxity (Lee et al., 2008). The most abundant lipid peroxidation product is 4-hydroxynonenal (4-HNE). In the study by Lee et al. (2008), RDH12 did not protect cells against 4-HNE. However, Marchette et al. (2010) showed that HEK-293 cells stably transfected with RDH12 did protect from 4-HNE induced cell death, and a greater amount of 4-HNE protein adducts accumulated in *Rdh12*$^{-/-}$ mice retinae following exposure to intense light.

RDH12 was also shown to convert dihydrotestosterone (DHT) to androstanediol, suggesting a possible involvement in steroid metabolism (Keller and Adamski, 2007). DHT has been implicated in the pathogenesis of androgenic alopecia (male pattern baldness) (Marchetti and Barth, 2013). However, no reports of this phenotype have been described in *RDH12* patients.

### 6. RDH12 activity of disease-associated mutants

A number of studies have utilised RDH12 transfected cell lines to study the enzyme activity of disease-associated mutants. In COS-7 cells transiently transfected with various *RDH12* missense mutants, 11 out of 14 variants showed significantly reduced enzyme activity, 5-18% of wild type levels. They also showed decreased expression levels, most likely as a result of protein instability (Thompson et al., 2005). Enzyme

activity was reduced by ~95% for p.(T49M), and p.(A269Gfs*2) variants. The p.(C201R) variant displayed no enzyme activity, consistent with the C201 residue being located in the highly conserved active site of RDH12 (Sun et al., 2007). Lee et al. (2007) studied the effect of 6 variants that resulted in substitutions in the cofactor or substrate binding sites, in Sf9 cells. In all cases, expression yields were lower for mutant proteins, and reduced activity was observed in 4 of the 6 mutants. Some discrepancy regarding the p.(T49M) variant is observed in the literature with Janecke et al (2004), Thompson et al (2005) and Lee et al (2007) reporting higher catalytic activity compared to wild type RDH12, however the study by Sun et al (2007) reported a reduction in enzyme activity of 95% compared to wild type. *RDH12* missense variants, p.(T49M) and p.(I51N), transiently transfected in HEK-293 cells, were shown to degrade at a faster rate than the wildtype protein with significantly lower half lives (Lee et al., 2010). Accelerated degradation of mutant protein may be a result of misfolding, resulting in the lower protein expression levels and subsequent reduced catalytic activity. Additionally, HEK-293 cells transfected with the p.(T49M) mutant lose their ability to protect against 4-HNE induced apoptosis (Marchette et al., 2010).

## 7. Overview of the visual cycle and the role of RDH12

RDH12 localises to the inner segments of the photoreceptors, where it functions as a retinal reductase, as part of the visual cycle. Light is absorbed by the visual pigments, rhodopsin and opsins, of the rod and cone photoreceptors, respectively. The light sensitive 11-cis retinal is covalently linked to the visual pigments. The first step in vision is the photoisomerisation of 11-cis retinal to all-trans retinal. For photoreceptors to function under constant light, all-trans retinal must be converted back to 11-cis retinal. This is achieved through a series of redox steps in the photoreceptors and RPE, collectively known as the visual cycle. All-trans retinal is reduced to all-trans retinol in the photoreceptor outer segments by RDH8, and in the inner segments by RDH12. All-trans retinol is transported to the RPE bound to

interphotoreceptor binding protein (IRBP). In the RPE, all-trans retinol undergoes oxidation and reduction, resulting in the regeneration of 11-cis retinal. 11-cis retinal is transported back to the photoreceptors bound to IRBP, where it combines with opsin again and is ready to be activated by a photon (Sahu and Maeda, 2016) (Figure 5). A second cone specific visual cycle also exists, where all-trans retinal is reduced to all-trans retinol. All-trans retinol is transported to the Müller cells, where it is isomerised to 11-cis retinol, this is then transported to the cone photoreceptors, where it is oxidised back to 11-cis retinal (Wang and Kefalov, 2011).

Studies on the *Rdh8*$^{-/-}$ *Rdh12*$^{-/-}$ double knockout mice, showed that Rdh8 accounts for 70% of all-trans RDH activity (Maeda et al., 2007). Using the intrinsic fluorescence of retinal and retinol, Chen et al. (2012) showed that all-trans retinal in mouse photoreceptors is reduced predominantly by Rdh8 and Rdh12. The majority of all-trans retinal is reduced by Rdh8 in the outer segments, but some all-trans retinal can leak into the inner segments, where it is reduced by Rdh12 (Chen et al., 2012). These studies suggest that the role of RDH12 in the visual cycle is minimal, but possibly plays a protective role in the clearance of all-trans retinal in periods of intense illumination.

Another possible role of RDH12 is protection against toxic lipid peroxidation products, like nonanal and 4-HNE, produced from the oxidative attack of polyunsaturated fatty acids in lipid membranes. A buildup of either all-trans retinal or lipid peroxidation products is damaging to photoreceptors. All-trans retinal accumulation leads to the production of toxic N-retinylidene-N-retinylethanolamine (A2E), and lipid peroxidation products are inherently toxic. RDH12 appears to have two possible roles, however it is unclear whether the build-up of all-trans retinal or the lack of protection against toxic lipid peroxidation products, is the primary cause of photoreceptor degeneration.

## 8. RDH12 animal models

The first *RDH12* animal model reported was the *Rdh12*$^{-/-}$ mouse model, which was generated by replacement of exons 1-3 of the *Rdh12* gene with a neomycin cassette (Maeda et al., 2006). *Rdh12*$^{-/-}$ mice displayed normal retinal morphology at 6 weeks of age. There was no significant difference in rhodopsin levels, indicating efficient regeneration of the chromophore. No difference in all-trans RDH activity in dissected retinae or isolated rod outer segments (ROS) between wildtype and *Rdh12*$^{-/-}$ mice was observed, suggesting that other enzymes may be compensating for the loss of Rdh12 activity. Knockout mice did however show a delayed dark adaptation and accumulation of all-trans retinal after bleaching, indicating an important role of RDH12 under conditions of excess illumination (Maeda et al., 2006). The second *Rdh12*$^{-/-}$ mouse model was reported by Kurth et al. (2007), and was created by targeted deletion of exons 1-3. *Rdh12*$^{-/-}$ mice were largely comparable to wildtype mice, with normal retinal histology until 10 months, and similar retinoid levels, with no signs of apparent retinal degeneration. However, retinal homogenates did show decreased all-trans retinal reduction, and increased A2E levels (Chrispell et al., 2009). Despite the severe phenotype observed in patients, the *Rdh12*$^{-/-}$ mouse models do not recapitulate this phenotype, and thus present several limitations for the study of RDH12 disease mechanisms.

*Rdh8*$^{-/-}$ *Rdh12*$^{-/-}$ double knockouts also showed mild light-dependent retinal degeneration, with delayed dark adaptation and reduced all-trans RDH activity with a build-up of all-trans retinal, and a subsequent accumulation of toxic N-retinylidene-N-retinylethanolamine (A2E) also observed (Maeda et al., 2007). However, *in vitro* experiments showed that Rdh8 and Rdh12 were responsible for >98% of all-trans RDH activity, with Rdh8 accounting for 70% of all-trans retinal clearance. Double knockout mice did however regenerate the visual pigment *in vivo* and triple knockout *Rdh8*$^{-/-}$, *Rdh12*$^{-/-}$, *Rdh5*$^{-/-}$ mice also had the ability to regenerate 11-cis retinal. RDHs do not appear to be necessary for the regeneration of the visual pigment in mice, but are needed for clearance of all-trans retinal in periods of excess illumination. It is

possible that mice RDHs compensate for each other. Other disease models are required to help shed light on the pathogenesis of *RDH12* retinopathy.

## 9. Therapeutic options

Without a representative model of *RDH12* related disease, the development of new therapies remains challenging. Very little research has been carried out into possible therapeutic avenues for patients with *RDH12* retinopathies. With the success of *RPE65* gene therapy clinical trials, there is hope for other LCA genes. Promising results from AAV-mediated *RDH12* gene replacement therapy in mice have been reported. Human *RDH12* packaged in an AAV2/5 vector (rAAV2/5-hGRK1p.hRDH12) was delivered to wildtype and *Rdh12*$^{-/-}$ mice by subretinal injection, resulting in correctly localized expression of RDH12, and maintained structural integrity of the retina. Retinal homogenates from injected mice showed increased all-trans retinol formation, with no visual cycle disruption and displayed decreased light damage susceptibility (Feathers et al., 2019).

In the absence of functional RDH12 protein, a build-up of free all-trans retinal ensues. Exposure to bright light and excessive all-trans retinal leads to the formation of toxic A2E, which is formed from two molecules of all-trans retinal and one molecule of ethanolamine, and is toxic to cells. A2E was shown to accumulate in the retinas of *Rdh12*$^{-/-}$ *Rdh8*$^{-/-}$ mice, leading to retinal degeneration (Maeda et al., 2007). In a study on *Rdh8*$^{-/-}$*Abca4*$^{-/-}$ mice, a panel of primary amine-containing FDA approved drugs were tested for their ability to lower levels of free all-trans retinal. Primary amines can react with all-trans retinal, forming transient conjugates that are eventually broken down and cleared, thereby preventing the formation of A2E. A number of primary amine drugs were protective against retinal degeneration, without inhibiting chromophore regeneration. One of the drugs, A20, a racemic mixture of 3-aminomethyl-5-

methylhexanoic acid, was shown to protect light induced retinal degeneration in *Rdh12*$^{-/-}$ mice (Maeda et al., 2011). These results provide a promising avenue for potential therapeutic intervention, although further studies are needed to determine the safety and effectiveness of these drugs in patients with retinal dystrophies.

Variants that cause protein misfolding can lead to accelerated degradation of the mutant protein. In HEK-293 cells transfected with various *RDH12* variants, lower expression levels of mutant protein were seen. Chemical chaperones can be used to promote protein folding, thereby increasing the stability of the protein and enhancing activity. Dimethylsulfoxide (DMSO) was shown to increase the stability and retinaldehyde reductase activity of the p.(T49M) *RDH12* variant, however, no similar increase was observed for the p.(I51N) variant, indicating the stabilising effect of chemical chaperones may be mutation specific (Lee et al., 2011a). Further work is needed to determine the effectiveness of in a range of different variants.

Therapies focused on preserving retinal function and slowing photoreceptor degeneration are increasingly being explored, that work independently of the mutation and gene, hence can be applied to a broad range of retinal dystrophies. Oxidative stress is a main contributor to cone degeneration. The antioxidant drug N-acetylcysteine (NAC) was shown to inhibit cone death by reducing oxidative damage in retinitis pigmentosa (RP) mouse models (Lee et al., 2011b) and a clinical trial is currently underway in RP patients (NCT03063021). Rod-derived cone viability factor (RdCVF) is secreted by rods, and promotes cone survival by aiding glucose uptake and metabolism (Ait-Ali et al., 2015). AAV-mediated delivery protects cones from degeneration in a mouse model (Byrne et al., 2015) and phase I clinical trials are planned for patients with RP.

## 10. Future Perspectives

Although considerable research has been carried out into the molecular genetics and biochemical characterisation of RDH12, there is still a significant lack of knowledge regarding the exact disease mechanism in humans and no treatments exist. It is unclear whether the build-up of all-trans retinal or the lack of protection against toxic lipid peroxidation products, is the primary cause of photoreceptor degeneration. Despite the severe phenotype displayed in patients with LCA13, the *Rdh12* knockout mouse models do not show a comparable phenotype.

A number of studies have utilised RDH12 transfected HEK-293 or COS-7 cells to study RDH12 activity, however these cells are not an accurate representation of the cells native environment. A more representative disease model is required to understand the impact of *RDH12* mutations in humans and to potentiate the development of novel therapies. It is now possible to generate 3D retinal organoids with functional photoreceptors from induced pluripotent stem cells, derived from a patients' own skin or blood sample. Retinal organoids have been shown to recapitulate human development and would provide the ideal model to study RDH12 in patients with varying mutations. Retinal organoids represent the future of disease modelling and development of personalised therapies for patients with a wide range of retinal dystrophies.

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**Conflict of interest statement**

The authors declare no competing interests.

**Figures**

Colour to be used for Figures 1, 3 and 5.

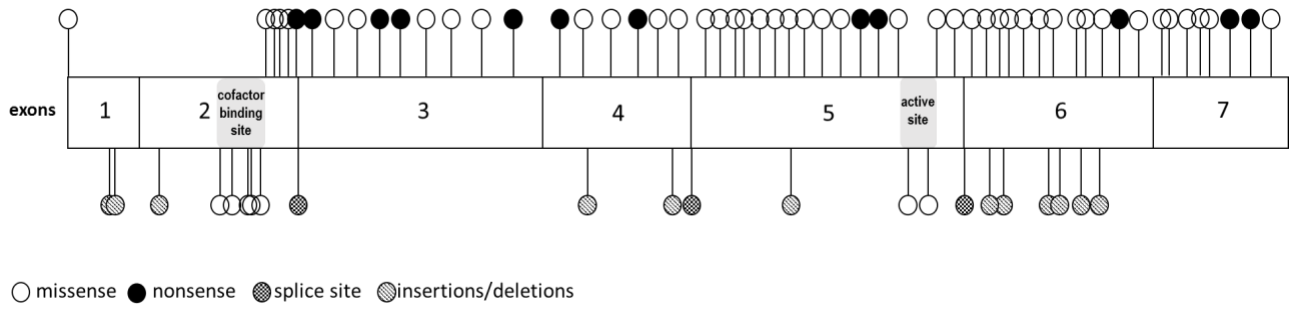


**Figure 1: Reported mutations for *RDH12*.** Data from Human Gene Mutation database (http://www.hgmd.cf.ac.uk/ac/gene.php?gene=RDH12 accessed April 2019)

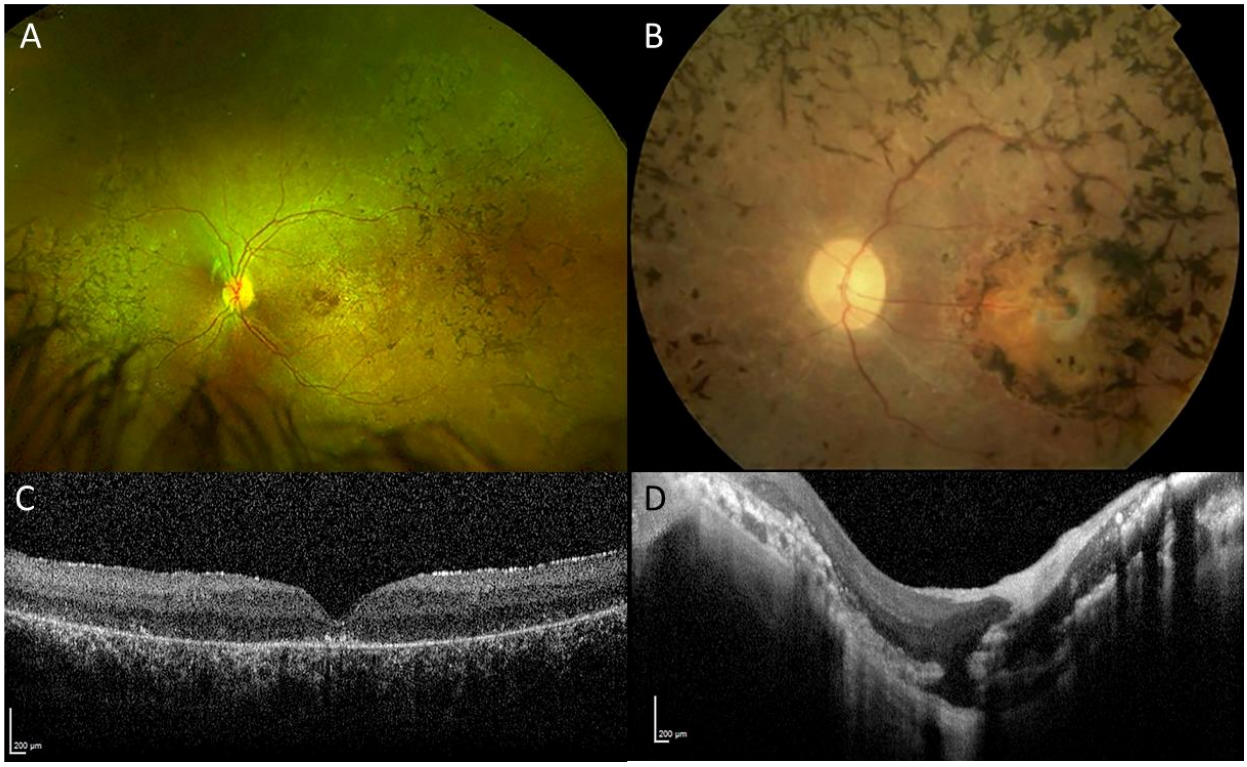


**Figure 2: Clinical phenotype of autosomal recessive *RDH12* LCA.** (A) Optos colour image of left fundus from a 4 year old girl with compound heterozygous missense mutations showing mid-peripheral bone spicules and macular atrophy with corresponding SD-OCT (B) confirming loss of the outer nuclear layer (ONL), ellipsoid zone and RPE. (C) Left fundus photo from a 43 year old lady with a homozygous missense mutation showing macular atrophy, extensive bone spicules and a waxy disc with retinal vessel attenuation; corresponding SD-OCT (D) displayed a posterior staphyloma and extensive macular atrophy and structural disorganisation.

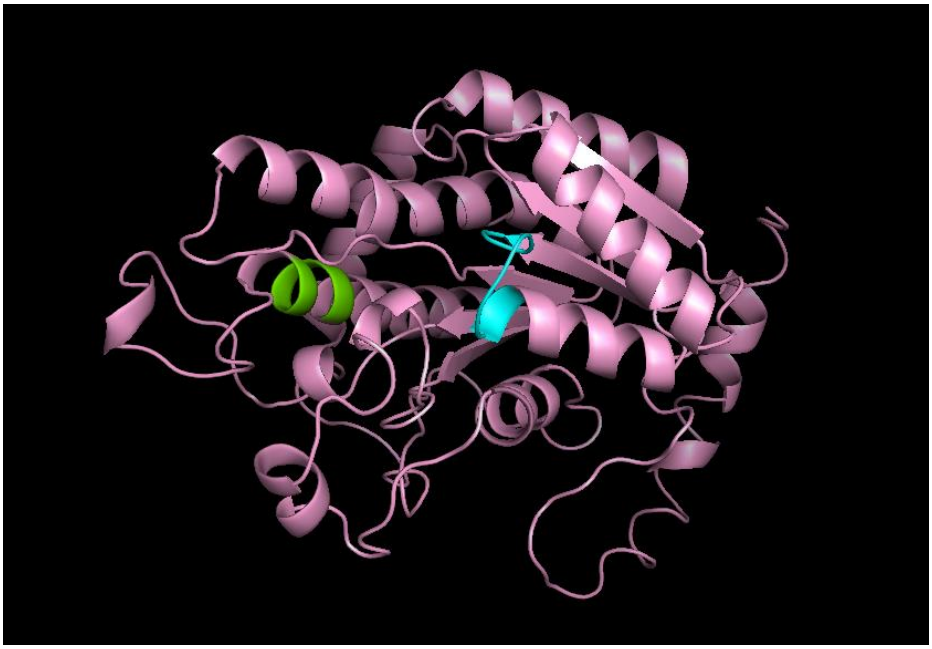

**Figure 3: Homology model of RDH12.** Model created using Phyre2 software (Kelley et al., 2015). Active site is shown is green and the cofactor binding site is shown in blue.

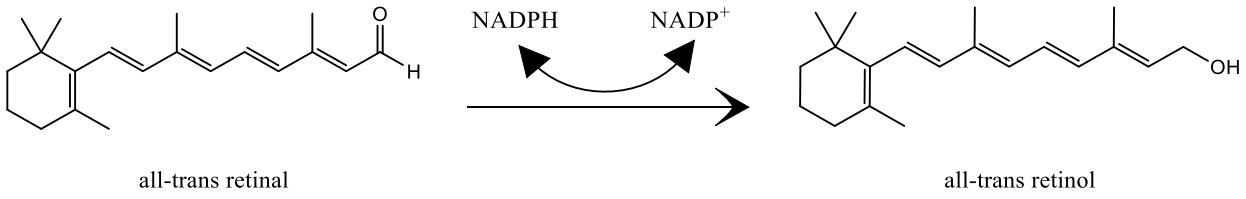


**Figure 4: RDH12 catalyses the reduction of all-trans retinal to all-trans retinol**.

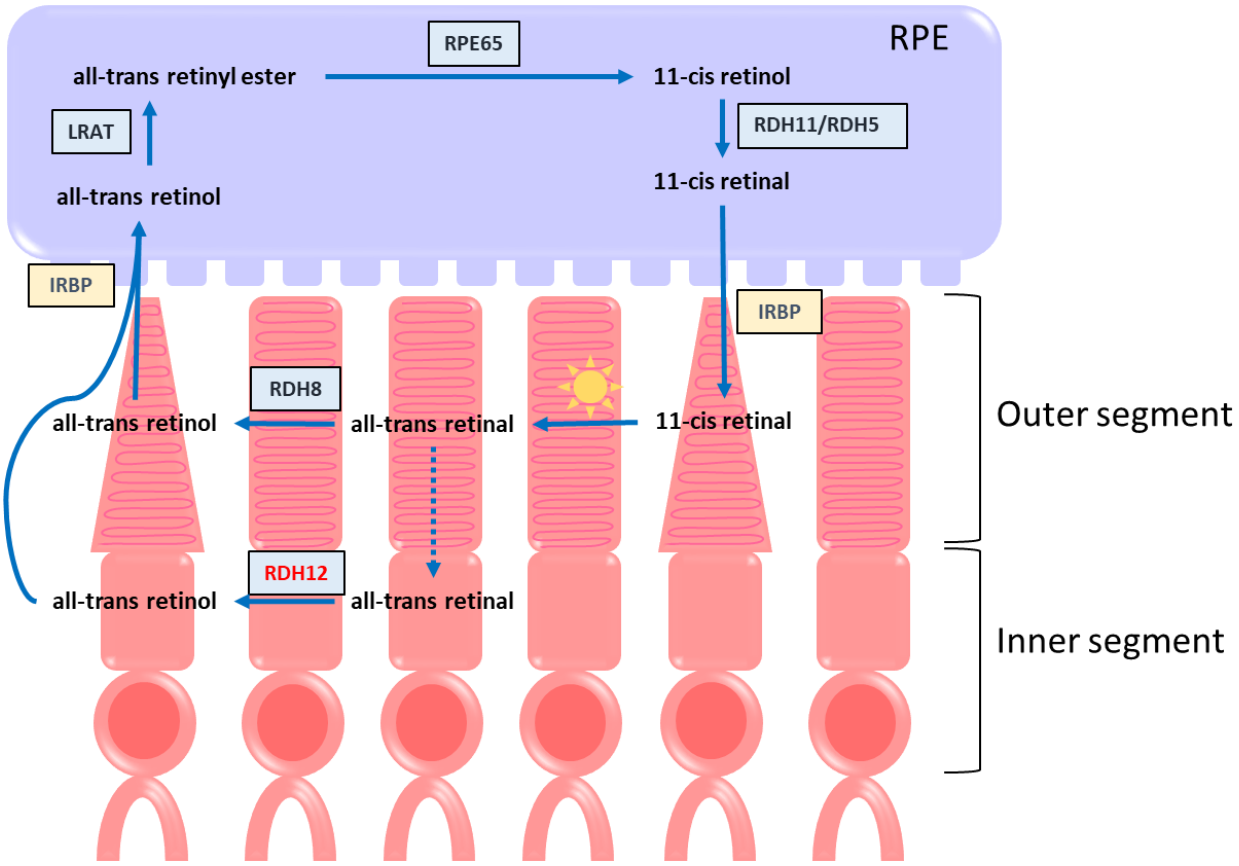


**Figure 5: Regeneration of the visual chromophore via the classical visual cycle.**

IPBP; Interphotoreceptor binding protein, LRAT; Lecithin retinol acyltransferase, RPE65; Retinoid Isomerohydrolase, RDH; Retinol dehydrogenase

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