1. Introduction

The cadherin superfamily encompasses a diverse group of cell adhesion molecules with crucial functions throughout the development and lifespan of complex organisms (El-Amraoui and Petit, 2013, Leckband and de Rooij, 2014, Priest *et al.*, 2017). The term cadherin was attributed to the family of molecules responsible for calcium-dependent cell-to-cell adhesion (Hatta and Takeichi, 1986). We focus here on the superfamily members essential for the development and function of the vertebrate retina, where cadherins contribute to tissue morphogenesis, neural circuit formation, neuronal survival, and photoreceptor development and maintenance. We give particular attention to the four cadherins encoded by genes known to be associated with inherited monogenic retinopathies: *CDHR1, CDH3, CDH23,* and *PCDH15*. Firstly, we review the classification of the cadherin superfamily and the roles of known cadherins in the outer and inner retina. Each monogenic retinal cadherinopathy is then described in detail, including its discovery, clinical phenotypes, gene structure and clinical genetics, and uncertainties relevant to each gene. Finally, we summarise the likely strategies towards molecular therapies for each retinal cadherinopathy.

1.1. Classification of the cadherin superfamily

The cadherins are a diverse superfamily of cell adhesion molecules that serve an array of essential roles, from facilitating structural aggregation and cell migration at adherens junctions to cell-cell signalling and planar cell polarity. There are over 100 members of the superfamily, predicated based on the presence of at least two extracellular cadherin (EC) repeats in tandem. An individual EC repeat is ~100 amino acids in length. They vary in sequence but feature seven β-strands organized in a "Greek key" topology (reviewed in (Shapiro and Weis, 2009, Sotomayor *et al.*, 2014)). Linker regions between EC repeats include three calcium binding sites, and the presence of Ca^{2+} ions is generally required for adhesive interactions. Various criteria have been used to classify superfamily members into subgroups with initial strategies focusing on the cytoplasmic domain sequence. This is

an evolving area, and as classifications have changed many superfamily members have been assigned new names. Recent classification schemes based primarily on the sequence of EC1, the EC repeat most distal to the membrane, divide the superfamily into two main groups: the cadherin main branch which includes classical cadherins, and the cadherin related branch, which includes protocadherins and atypical cadherins (Figure 1) (Sotomayor et al., 2014, Hulpiau and van Roy, 2009). We will use current nomenclature and mention prior aliases.

1.1.1. Classical cadherins

Cadherin-1 (CDH1, formerly E-cadherin, or epithelial cadherin) was the first member of the superfamily, identified by Yoshida and Takeichi as the mediator of Ca^{2+}-dependent cell adhesion in a teratocarcinoma cell line (Yoshida and Takeichi, 1982). CDH1 is an exemplar of the classical cadherins, which contain five EC repeats and a single transmembrane domain. Classical cadherins engage in homophilic *trans*-interactions between cell membranes or heterophilic interactions primarily with other classical cadherins (Boggon *et al.*, 2002, Harrison *et al.*, 2011). *Trans* binding is mediated by EC1 in a mechanism involving swapping of the N-terminal β strands between the two monomers, resulting in a very stable adhesive interaction (Boggon et al., 2002). These molecules can also engage in extracellular *cis* interactions between molecules side-by-side outside the cell membrane (Yap *et al.*, 1997, Harrison et al., 2011). Classical cadherins interact directly with β-catenin and p120-catenin through their cytoplasmic domain. β-catenin associates with the cytoplasmic domain beginning in the endoplasmic reticulum and is necessary for cadherin localization to the plasma membrane (Chen *et al.*, 1999), while p120-catenin stabilizes cadherin at the cell surface by preventing its endocytosis and degradation (Ishiyama *et al.*, 2010). β-catenin recruits α-catenin which provides an essential link to the actin cytoskeleton through interaction with an array of actin binding molecules and potentially directly with actin filaments (Figure 2) (Aberle *et al.*, 1996). Clusters of cadherin/catenin complexes with stable actin filaments comprise adherens

junctions and are essential for maintaining tissue structural integrity. Adherens junctions contain ~700-1200 cadherin molecules per μm^2 (reviewed in (Harris and Tepass, 2010)).

Classical cadherins are further divided based on the presence (Type-I) or absences (Type-II) of a conserved His-Ala-Val motif within EC1. Type-I and -II cadherins also differ at their adhesive interfaces, as either one (Type-I) or two (Type-II) conserved tryptophan residues at the N-terminus of the mature protein are essential for stable *trans* interactions. CDH3 (formerly P-cadherin or placental cadherin), discussed below, is a Type-I classical cadherin. The cadherin main branch (Sotomayor et al., 2014, Hulpiau and van Roy, 2009) also includes the following: desmocolins and desmogleins, which mediate adhesion at desmosomes, adhesive junctions linked to intermediate filaments such as keratin filaments rather than the actin cytoskeleton as are adherens junctions (Harmon and Green, 2013, Yin and Green, 2004); the 7D cadherins, which have specialized roles in cells involved in water transport in the intestine, liver, and kidney (Weth *et al.*, 2017), and the seven-transmembrane CELSRs which are involved in planar cell polarity and axon guidance throughout the nervous system (Figure 1) (Goffinet and Tissir, 2017).

*1.1.2. Protocadherins and cadherin related molecule*s

Protocadherins (PCDHs) were first discovered in a search for additional classical cadherins by degenerate PCR (Sano *et al.*, 1993). PCDHs have 6 or 7 extracellular EC repeats and divergent cytoplasmic domains that do not interact with catenins. These include the ~60 clustered PCDHs which have 6 EC repeats and are encoded by three gene clusters arrayed in tandem on human chromosome 5q31 and mouse chromosome 18 (α, β, and γ- PCDHs encoded by *PCDHA*, *PCDHB*, and *PCDHG* respectively) (Wu and Maniatis, 1999). α- and γ- PCDHs each have subfamily-specific constant domains encoded by shared exons, while β-PCDHs are encoded by individual exons and have no constant domain. The unclustered δ-PCDHs are divided into two groups, δ1 with 7 EC

repeats and δ2 with 6 (Redies *et al.*, 2005). Protocadherins are broadly expressed in the nervous system and serve a host of essential roles in neural development and function.

The atypical cadherin related molecules range from the Calsyntenins (CLSTN1-3) with only 2 EC repeats (Hintsch *et al.*, 2002) to the FATs (FAT1-3) with 34 EC repeats, orthologues of *Drosophila fat*, so named for its role in regulating organ size (Blair and McNeill, 2018, Aviles and Goodrich, 2017). This more loosely defined category includes three members encoded by genes linked with monogenic retinopathies: CDHR1 with 6 EC repeats, CDH23 with 27 EC repeats, and PCDH15 with 11 EC repeats (Figure 1).

2. *CDHR1* and retinal degeneration

2.1 - Identification of human CDHR1-associated retinal degeneration

Following the description of a photoreceptor specific cadherin (prCAD) that was essential for normal rod and cone photoreceptor function in 2001, it was suggested in 2005 that the human prCAD orthologue, *CDHR1*, also known as *PCDH21*, was a candidate gene for human retinal degeneration (Bolz *et al.*, 2005). The authors described the genomic structure of the human gene, and although a few sequence variants were found, biallelic variants could not be identified in a cohort of patients with retinitis pigmentosa, Leber's congenital amaurosis and Usher syndrome (Bolz et al., 2005); at the time, the retinal phenotype was not known. In 2010, two near co-incident reports identified biallelic variants in *CDHR1* as a cause of retinal degeneration (Ostergaard *et al.*, 2010, Henderson *et al.*, 2010). The first submitted report originated from the Faroe Islands in which there is a relatively high prevalence of recessive genetic disorders. Autozygosity mapping in a family with 6 affected individuals with cone-rod dystrophy identified a truncating variant in *CDHR1* which segregated with the disease (Ostergaard et al., 2010). Two further families of South Asian and Middle Eastern descent with

autosomal recessive retinal degeneration were found on autozygosity mapping to harbour two different truncating variants in *CDHR1* (Henderson et al., 2010). In both studies, the previously reported phenotype of the *Cdhr1*$^{-/-}$ mouse supported the designation of *CDHR1* as the causative gene in pedigrees with evidence of both rod and cone dysfunction.

2.2 - Pathological sequence variants in CDHR1

Following the original identification of *CDHR1*-associated retinal degeneration, around 20 subsequent clinical reports have described further individuals and pedigrees. Supplementary Table 2 lists all reported and clinically validated pathological variants in *CDHR1* with their protein consequence, mean allele frequency, associated retinal phenotype and reference. The position of the variants is illustrated against the CDHR1 protein in Figure 4.

The *CDHR1* gene spans 17 exons with a transcript length of 2,580bp (Table 1). The translated CDHR1 protein is a non-classical cadherin with 6 extracellular cadherin-like repeats, a transmembrane domain and cytoplasmic/intracellular domain (Figures 1&2). The extracellular domain is more highly conserved than the cytoplasmic domain (63.4% sequence identity versus 31.2%), suggesting a more important biological function, where these three proteins are likely to interact (Supplementary Table 1). Only a single clinically-validated missense variant (p.Pro735Thr) has been reported in the 138 codons long cytoplasmic domain of CDHR1, compared to a missense variant on average every 86 codons in the ectodomain. This observation further supports the hypothesis of an important biological function for the ectodomain. The cytoplasmic domain, whilst poorly conserved in general, bears several clusters of homology, and the above-mentioned single disease-associated variant is likely hypomorphic. It is possible that these regions are required to interact with an as yet unidentified intracellular protein, possibly to stabilise CDHR1 within the plasma membrane, given that its structural role in disc morphogenesis appears compelling (Burgoyne *et al.*, 2015).

2.3 - Clinical phenotype in patients with biallelic null variants in CDHR1

Aggregation of the combined allele frequencies of pathological variants in characterised ethnic populations across the world with application of the Hardy-Weinberg equilibrium enables an approximation of the number of affected individuals and carriers with recessive disease. Using this methodology, more than 10,000 individuals have been predicted to harbour biallelic null variants in *CDHR1* (Hanany *et al.*, 2020). However, this value is likely to represent an underestimate as missense variants in *CDHR1* were excluded from the analysis (Supplementary Table 2). Null variants in this section refers to patients with start loss, stop gained, frameshift variants and those affecting canonical splice sites in *CDHR1*. Table 2 compares the clinical features of the four retinal cadherinopathies to which the descriptive texts provides additional details.

Symptoms

In aggregated clinical data from 26 patients with biallelic null variants in *CDHR1*, the most common presenting symptom was nyctalopia (54%), followed by photoaversion (39%), decreased vision (31%), dyschromatopsia (19%), and decreased peripheral vision (8%) (Bessette *et al.*, 2017). The onset of night blindness is typically described in childhood or early adulthood, indicating rod dysfunction (Ostergaard et al., 2010, Henderson et al., 2010, Duncan *et al.*, 2012). The onset of symptoms attributable to cone dysfunction (glare, photophobia) is variable: with some case series describing the onset at presentation (Ba-Abbad *et al.*, 2013), in childhood (Duncan et al., 2012), or later (Cohen *et al.*, 2012, Ostergaard et al., 2010).

Colour vision, where tested, appeared to be severely reduced or absent (Duncan et al., 2012, Cohen et al., 2012, Ba-Abbad et al., 2013), and the age at which dyschromatopsia was reported was variable (Duncan et al., 2012). The onset of symptoms related to cone dysfunction appear sooner and with a

greater severity that can be expected in a primary rod degeneration in which the involvement of cones is typically later, by a secondary mechanism.

Clinical signs

Fundal examination may reveal signs at the macula - mottling, or irregular beaten-metal appearance - with later, variable peripheral retinal pigment migration, arteriolar attenuation, scalloped RPE atrophy anterior to the arcades, peripheral retinal degeneration with or without waxy optic disc pallor as typically seen in RP (Henderson et al., 2010, Ostergaard et al., 2010). Macular atrophy appears to be near-universal and progressive, resembling a bull's eye configuration or geographic atrophy in late disease, with or without pigmentation, and was identifiable in 96% of individuals in aggregated data of reported cases *(n=26)*(Bessette et al., 2017). Visual acuity appears to be variably affected (Bessette et al., 2017); the natural history of visual acuity decline has not been well characterised. However, several reports indicated relative preservation of visual acuity until the 4-5th decade when macular atrophy involved the fovea (Henderson et al., 2010, Duncan et al., 2012). High myopia may be observed in some individuals (Duncan et al., 2012).

Peripheral visual field findings appear to be variable; some affected individuals demonstrate severe concentric visual field constriction (Nikopoulos *et al.*, 2015, Duncan et al., 2012) with some retaining islands of vision in late disease (i.e. temporally as seen in retinitis pigmentosa). Central scotomas, or absent central responses can be identified as macular atrophy progresses (Duncan et al., 2012, Ba-Abbad et al., 2013). In early disease, microperimetry may identify abnormal thresholds at the posterior pole in the absence of atrophy, suggesting significant functional abnormalities of cone photoreceptors (Charbel Issa *et al.*, 2019).

Multimodal retinal imaging

Fundus autofluorescence imaging may show hyperautofluoresence within the macular arcades (Figure 4). Patchy or scalloped areas of RPE loss in the retinal periphery may coalesce, leading to widespread RPE loss. Peripapillary loss of autofluorescence is seen in some individuals but is not universal. In the macula, an abnormal foveal hypoautofluorescent signal is seen (Figure 4), which may appear ovoid or horizontally elongated. RPE loss develops within the macula, often sparing the fovea initially, with centrifugal enlargement. Generalised hyperautofluorescence may be seen at the posterior pole (Figure 4), and increased quantitative fundus autofluorescence has been identified in *CDHR1*-related retinopathy, indicating increased lipofuscin accumulation (Gliem *et al.*, 2020).

OCT imaging in early disease may reveal significant generalised outer nuclear layer thinning with loss ellipsoid zone peripherally and loss of continuity centrally. OS appear shortened and the foveal interdigitation zone may be lost earlier than expected in a rod-cone dystrophy (Figure 4). As disease progresses, the distinction between outer retinal laminae becomes less obvious with loss of the ellipsoid and interdigitation zone. RPE loss may be evident at the macula. Adaptive optics scanning laser ophthalmoscopy in a single individual examined using the technique showed increased cone spacing, although cone reflectivity was absent where ellipsoid zone abnormalities were seen (Duncan et al., 2012). This does not however necessarily indicate the absence of cones.

Electrophysiology

Full-field ERG testing is characterised by attenuation of cone and rod responses in all individuals. In early disease, visual function as determined by ERG may be affected disproportionately to the clinical appearances and retinal imaging studies (Ba-Abbad et al., 2013). Although both attenuated rod and cone responses could be identified in some patients into the 5th decade, often they are severely attenuated or absent (Henderson et al., 2010, Duncan et al., 2012, Ba-Abbad et al., 2013). Rod

responses may be lost prior to cone responses(Cohen et al., 2012) or vice-versa (Ba-Abbad et al., 2013).

Carriers of a single *CDHR1* null variant have demonstrated normal visual acuity, colour vision, visual field examination and normal age-adjusted rod and cone responses on electroretinography (Duncan et al., 2012). These observations are consistent with observations of normal retinal structure and function in the *Cdhr1*$^{+/-}$ heterozygous mouse.

2.4 - *Clinical phenotype in patients with CDHR1-related late-onset macular dystrophy*

An additional macula-predominant retinal phenotype was described in individuals with biallelic variants in *CDHR1* (Charbel Issa et al., 2019, Stingl *et al.*, 2017). The significance of this phenotype is that the later age of onset and progressive macular atrophy may be confused with geographic atrophy due to age-related macular degeneration and consequently be under-diagnosed. Moreover, the finding established *CDHR1* as a recessive cause of central areolar choroidal dystrophy; other genetic causes of this phenotype result in dominant inheritance (specific monoallelic missense variants in *PRPH2*, *GUCY2D*, *GUCA1A*).

Late-onset macular dystrophy with otherwise relatively preserved retinal morphology and function does not appear to occur in individuals with biallelic null variants in *CDHR1* who have more severe, generalised rod and cone dysfunction as described above. Patients with late-onset macular dystrophy harbour at least one hypomorphic *CDHR1* allele (as indicated in Supplementary Table 2), although may exist with variable mild peripheral retinal degeneration depending on the functional effect of the second allele (Charbel Issa et al., 2019).

Symptoms

Patients with *CDHR1*-related late-onset macular dystrophy typically do not become symptomatic until the fourth decade. This was identified in individuals homozygous for the c.783G>A variant, common in European populations (mean allele frequency of 0.49%) who presented with reduced visual acuity, difficulty in reading, glare, poor contrast and metamorphopsia (Charbel Issa et al., 2019). The symptom complex appeared similar in individuals with different combinations of *CDHR1* alleles (Ba-Abbad *et al.*, 2020). Typically, this group does not report nyctalopia (Charbel Issa et al., 2019) although this may be the case if a hypomorphic variant exists *in trans* with a more severe variant.

Visual acuity in this group is typically well preserved in at least one eye until later life, in contrast to individuals with biallelic null variants in *CDHR1*. Moreover, relative preservation of foveal cones may result in retention of a central island of retinal tissue which, when lost, results in a rapid decline in visual acuity (Ba-Abbad et al., 2020, Charbel Issa et al., 2019).

Clinical signs

The earliest clinical sign in this patient group is a maculopathy with a bull's eye distribution, which may be composed of yellow flecks/dots, or mild pigmentary changes (Ba-Abbad et al., 2020). With disease progression, RPE atrophy progresses to form a contiguous region of sharply demarcated RPE and photoreceptor loss that may resemble central areolar choroidal dystrophy. Yellow flecks may be present on the border of the atrophic region in some patients (Ba-Abbad et al., 2020, Charbel Issa et al., 2019). Peripapillary atrophy may be present in some cases which may coalesce with the area of macular atrophy (Ba-Abbad et al., 2020, Charbel Issa et al., 2019). The fovea appears spared until relatively late in many cases. The peripheral retina appears unaffected in most patients although may be involved in individuals with a null variant on the second allele.

Multimodal retinal imaging

Fundus autofluorescence imaging may reveal yellow flecks/dots as punctate hyperautofluorescent dots which may be distributed in a bull's eye pattern interspersed with mottled hypoautofluorescent regions. Areas of retinal atrophy coalesce and progressively enlarge with hyperautofluoresecent signal from the border of sharply demarcated RPE and photoreceptor loss (Figure 4) (Charbel Issa et al., 2019, Ba-Abbad et al., 2020). The fovea appears relatively preserved, and may survive as an island or continuous with the retina outside the area of atrophy through a thin connecting stalk before it is lost and visual acuity declines sharply.

Optical coherence tomography imaging demonstrates an irregularity in the paracentral ellipsoid zone and RPE bands in early disease with relative preservation of the fovea. The outer nuclear layer appears thin over the affected areas. In late disease, there is near complete loss of the outer nuclear layer and deeper retinal layers, leaving inner retinal layers over Bruch's membrane. However, the retina at the border of the lesion may appear normal depending on the *CDHR1* variants (Charbel Issa et al., 2019, Ba-Abbad et al., 2020). Transmission effects can be seen in regions of advanced atrophy where the RPE is lost. Outer retinal morphology appears to be spared at beyond the limits of RPE loss on OCT imaging, unless a null(-like) variant is present on the second allele.

Visual field

Full field testing identified normal peripheral thresholds on kinetic perimetry where tested in individuals with biallelic hypomorphic *CDHR1* alleles (Charbel Issa et al., 2019). Furthermore, microperimetry in patients with two hypomorphic *CDHR1* variants identified relatively normal retinal function beyond the area of RPE/retinal atrophy, in contrast to patients with one or more truncating variants where peripheral retinal function was impaired (Charbel Issa et al., 2019).

Electrophysiology

In patients homozygous for the c.783G>A allele, full-field electroretinography demonstrated either normal examinations or a mild reduction of photopic versus scotopic responses. This was confirmed in a further study with compound heterozygous presumed hypomorphic variants, where affected patients exhibited reduced amplitudes on light-adapted versus dark-adapted tests indicating a more significant functional effect on the cone system (Ba-Abbad et al., 2020). In patients with hypomorphic variants in *trans* with a truncating or more severe variant, functional losses on ERG appeared to be more significant with cones affected more than rods (Charbel Issa et al., 2019). Pattern ERG P50 response is unrecordable in most patients with late-onset macular dystrophy with delayed or reduced P50 responses in earlier disease indicating macular dysfunction (Ba-Abbad et al., 2020). Multifocal ERG were universally subnormal with relative preservation of foveal responses and eccentric responses when compared to those in the parafovea (Ba-Abbad et al., 2020).

3. *CDH23* causing Usher syndrome 1D

Usher syndrome is the most common cause of combined deaf-blind dual sensory impairment. Usher syndrome is categorised into 3 types according to clinical features which are linked to specific loci and genes. Usher syndrome Type 1 (USH1) is characterised by profound congenital hearing loss, vestibular areflexia and retinitis pigmentosa with onset around puberty (Reiners *et al.*, 2006). Usher syndrome type 2 (USH2) is associated with moderate to severe hearing impairment with normal vestibular function and variable but later onset of RP compared to USH1. Usher type 3 is rare, and associated with progressive hearing loss and variable retinal and vestibular dysfunction.

USH1 is genetically heterogenous with 6 associated genes currently identified. The most common cause of USH1 are variants in *MYO7A* (USH1B; 39-48%) (Oshima *et al.*, 2008). It is estimated that approximately 20% of patients are attributable to variants in *CDH23* (USH1D). There are no

consistent clinical differences between the subtypes of Usher syndrome type 1; they are distinguished based on genetic analysis. A further common theme in USH1 is the presence of an associated autosomal-recessive non-syndromic deafness (DFNB) phenotype, typically associated with hypomorphic alleles. Numerous mouse models are available for these identified USH1 subtypes: USH1B, 1C, 1D, 1F, 1G, although none of the knock-out mouse models demonstrate clear evidence of retinal degeneration that recapitulates the human USH1 phenotype.

3.1 - Identification of *CDH23* variants in USH1D

USH1D was mapped to the long arm of chromosome 10 in 1996 through autozygosity mapping in a consanguineous pedigree with retinitis pigmentosa and sensorineural hearing loss (Wayne *et al.*, 1996). The identified locus overlapped with the locus attributed to DFNB12, a cause of recessive non-syndromic hearing loss (Chaib *et al.*, 1996). Three near co-incident and independent reports in 2001 identified *CDH23* as the gene underlying USH1D within this region (Bolz *et al.*, 2001, Bork *et al.*, 2001, Di Palma *et al.*, 2001a). Bolz *et al* used a positional candidate approach to identify *CDH23* in a pedigree with USH1D (Bolz et al., 2001). Bork *et al* used autozygosity mapping in a single family to identify a genomic interval in which truncating mutations in *CDH23* were found to cause USH1D and missense variants found to underlie DFNB12 in seven pedigrees (Bork et al., 2001). Di Palma *et al* identified truncating variants in three strains of waltzer (v) mice, affecting expression of *CDH23* in the neuroepithelium. Effects on hair cell differentiation and organization helped to establish *v* mice as the mouse model of human USH1D (Di Palma et al., 2001a). These three reports, published within the same year, appeared to mutually validate the conclusions of the other studies: that *CDH23* was the gene underlying USH1D and DFNB12 within the identified 10q22.1 locus.

3.2 - *CDH23* gene structure and disease-associated variants

CDH23 is located on the long arm of chromosome 10, encoding a 3,354 amino acid protein with 27 extracellular cadherin domains, a single-pass transmembrane domain and cytoplasmic domain (Figure 1). Nonsense, frameshift, splice-variants and certain missense variants – presumably which function as null alleles - in *CDH23* have been associated with USHID, whereas only missense variants have been associated with DFNB (Figure 5 and Supplementary Table 3). Interestingly, compound heterozygous individuals with one *CDH23* variant in *trans* with a milder DFNB12 variant have preserved vision and balance, indicating that little remaining *CDH23* function is sufficient to protect against retinal degeneration (Schultz *et al.*, 2011).

The extracellular domains of *CDH23* appear similar to those of E-cadherin which has a well characterised function in homophilic cell-to-cell adhesion through the formation of dimers from opposing cell surfaces (Astuto *et al.*, 2002). The extracellular domains contain cadherin-specific domains such as LRDE, DXD, DXNDN required for calcium binding and cadherin dimerization. It has been noted that missense *CDH23* variants underlying RNSD occur more frequently in these strongly-conserved extracellular calcium binding motifs, and rarely in USH1D patients, suggesting that calcium-dependent cell adhesion may be more important in the cochlear than in the retina (Astuto et al., 2002). However, this genotype-phenotype association is incomplete; at least 20 missense variants in *CDH23* are associated with USH1D (Supplementary Table 3) including a missense variant (p.Asn1098Ser) in the DXNDN calcium-binding motif (Oshima et al., 2008).

3.3 - Clinical phenotype

Usher syndrome type 1 (USH1) is characterised by profound, usually congenital deafness, absent bilateral vestibular function (the majority manifesting as delayed ambulation 18-36 months of age), and retinitis pigmentosa which becomes symptomatic by adolescence. The clinical presentation of retinitis pigmentosa (RP) appears to be typical in USH1D with symptoms dominated by progressive

rod dysfunction (nyctalopia, subjective visual field constriction), and is complicated in late disease by cone involvement. RP is characterised clinically by mid-peripheral pigment migration, arteriolar attenuation, waxy optic disc pallor and RPE loss which progresses centripetally. A hyperautofluorescent ring may be present which represents the point at which photoreceptor OS are lost and IS are retained, often seen in retinitis pigmentosa (Figure 5). In early disease, the macula may appear structurally normal on OCT imaging (Figure 5). The clinical phenotype of retinitis pigmentosa in USH1D patients demonstrates heterogeneity consistent with the variability of phenotypes affecting hearing and balance (Astuto et al., 2002, Pennings *et al.*, 2004). In one study of 37 patients with biallelic *CDH23* variants, 26 demonstrated extinguished ERG traces and 8 had subnormal ERG traces (Astuto et al., 2002). Visual field testing typically reveals severe constriction at more advanced ages, often with an additional persistent residual temporal island of vision in late disease as typically observed in RP. Colour vision may be normal. Posterior subcapsular cataract formation may be evident as seen in other forms of RP (Pennings et al., 2004).

Patients with DFNB exhibit moderate (40-60dB) to profound hearing loss (>90dB) that is usually congenital with rare exceptions. Moreover, the severity of hearing loss may be variable within pedigrees and progress at different rates. However, audiology identified a more severe hearing loss in patients with USH1D than DFNB12 (Pennings et al., 2004). There is no evidence of vestibular dysfunction in DFNB with normal age of ambulation (<12 months). Although patients with DFNB do not have symptoms of RP, in one series, 3 of 5 patients had subnormal ERG examinations (at 9-29 years of age), and 3 of 4 had abnormal fundus findings. However, all had normal visual field examinations (Astuto et al., 2002). It is likely that USH1D and DFNB due to *CDH23* variants form a phenotypic spectrum with hair cells in the inner ear being more susceptible to perturbations in CDH23 when compared to those in the vestibular apparatus or the retina which are not

symptomatically affected in DFNB patients. The finding of night blindness in a single pedigree with RNSD suggests unknown modifiers that may affect retinal function.

A single heterozygous missense variant (p.Arg1379Leu) in *CDH23* has been shown to segregate with familial pituitary adenoma (Zhang *et al.*, 2017). This variant has not been associated with USH1D in independently published cases (Figure 5). Moreover, detailed retinal phenotyping was not undertaken in this pedigree. There is insufficient evidence to recommend neuroimaging in patients with USH1D or DFNB12 associated with variants in *CDH23*, but this may require further clarification.

4 *PCDH15* causing Usher syndrome 1F

4.1 Identification of *PCDH15* variants in Usher syndrome type 1

Pcdh15 was first identified as the gene underlying the Ames waltzer (*av*) murine phenotype who exhibit profound deafness with vestibular dysfunction, manifesting as circling behaviour (Alagramam *et al.*, 2001a). In *av* mice, probes against the regions flanking the previously unknown transgene insertion site were used to identify sequences from mouse cDNA libraries, identifying two exons from which the remainder of the gene was sequenced (Alagramam et al., 2001a). The gene contained 11 putative cadherin repeats, and a cytoplasmic domain with no homology with other cadherins described; it was designated a protocadherin, *Pcdh15* (Alagramam et al., 2001a). Further analysis confirmed *Pcdh15* expression in the brain and inner ear, although retinal expression was not specifically examined. Scanning electron microscopy of $Pcdh15^{-/-}$ homozygotes demonstrated abnormal stereocilia, consistent with the *av* phenotype.

The human orthologue of *Pcdh15* was mapped to 10q21-22 in region of synteny to mouse chromosome 10 where four loci associated with human hearing loss had been mapped, including

USH1F (Alagramam et al., 2001a). The human *PCDH15* gene was identified, sequenced, and two families segregating USH1F were found to have variants in *PCDH15*, a finding replicated in a further report (Ahmed *et al.*, 2001, Alagramam *et al.*, 2001b). In the first study, linkage analysis refined the recombination interval to 1cM within 10q21.1, overlapping the *USH1F* locus interval. In the second study, mouse Pcdh15 cDNA probe was used to screen a human cDNA library from which the human *PCDH15* sequence was identified. Importantly, northern blot analysis identified PCDH15 expression in the human retina to support the clinical association of retinal degeneration in USH1F. However, various *PCDH15* transcripts were identified in human retinal tissue in two studies, raising the possibility of alternatively spliced isoforms (Ahmed et al., 2001, Alagramam et al., 2001b).

4.2 - *PCDH15* gene structure and disease-associated variants

Biallelic truncating variants, or severe missense variants in *PCDH15* result in Usher syndrome type 1 (USH1F), whereas hypomorphic missense variants are associated with non-syndromic deafness (DFNB23). The basis of this genotype-phenotype association may be that hair cells in the inner ear appear more susceptible to perturbations in PCDH15 function than the retina. Disease-associated variants in *PCDH15* are listed in Supplementary Table 4. No specific mutational hotspots have been identified in the gene (Jaijo *et al.*, 2012). However, missense variants in *PCDH15* appear to predominantly affect the first two cadherin ectodomains (Figure 6).

PCDH15 variants underlie approximately 11-19% of Usher syndrome type 1 cases, and are therefore less common than *MYO7A* or *CDH23*. However, a prominent founder mutation in *PCDH15* in the Ashkenazi Jewish community (Arg245Ter) is responsible for around 50-60% of Usher syndrome type 1 cases in that population (Ben-Yosef *et al.*, 2003). The large intronic and non-coding exonic regions make *PCDH15* susceptible to large deletion and rearrangements due to breakpoints within the gene (Le Guedard *et al.*, 2007). Intragenic rearrangements in *PCDH15* may constitute up to 13%

of disease-associated variants in one series (Aller *et al.*, 2010). Screening for *PCDH15* variants, or testing for unsolved USH1 cases may therefore include a method of analysis (such as multiplex ligation-dependent probe amplification) which can detect large gene rearrangements.

Screening of 14,000 individuals for *PCDH15* sequence variants identified 52 individuals with truncating variants in the cytoplasmic domain, some of which were found to have a relatively high allele frequency (exon 33) (Perreault-Micale *et al.*, 2014). Truncating variants in this region may be tolerated because of the alternatively spliced isoforms in the cytoplasmic domain, because some transcripts may escape nonsense mediated decay and/or because the cytoplasmic domain is functionally redundant. This latter hypothesis may be supported by the poor sequence conservation in the cytoplasmic domain when compared to the extracellular domain in *PCDH15* (Supplementary Table 1). Moreover, studies of the Pcdh15 ortholog in Drosophila, Cad99c, found that loss of the cytoplasmic domain is well tolerated, suggesting that ectodomain length determines microvillus length (D'Alterio *et al.*, 2005). However, individuals with USH1F have been identified with truncating variants in the proximal cytoplasmic domain (Supplementary Table 4). The ectodomains of PCDH15 are critical to the formation of heterophilic tip-links between stereocilia with CDH23, gating mechanotransduction within the inner ear. The role of the cytoplasmic domain of PCDH15 is less clear although it may link to the actin cytoskeleton through either whirlin/MYO15A(Belyantseva *et al.*, 2005) or harmonin/MYO7A complex proteins (Figure 2). Missense variants in the cytoplasmic domain of *PCDH15* have not been associated with USH1F or DFNB23.

The evidence for digenic inheritance of *CDH23* and *PCDH15* is summarised in section 6. Non-syndromic hearing loss has been shown to be inherited in a digenic fashion with missense variants in PCDH15 and USH1G, following its description in digenic heterozygous mice (Schrauwen *et al.*,

2018). Numerous other digenic inheritance patterns of hearing loss involving USH1 genes has been reported, summarised in (Whatley *et al.*, 2020).

4.3 - Clinical phenotype

Patients with biallelic variants in *PCDH15* exhibit typical retinitis pigmentosa that is clinically indistinguishable from other forms of USH1, whose retinal phenotype is summarised in section 3.3. Numerous clinical reports are available which highlight the clinical features of individual cases, with data from electroretinography (Ahmed et al., 2001, Alagramam et al., 2001b, Ahmed *et al.*, 2003, Brownstein *et al.*, 2004, Le Guedard et al., 2007, Ahmed *et al.*, 2008, Chen *et al.*, 2015, Saleha *et al.*, 2016, Ben-Yosef et al., 2003, Jaijo et al., 2012).

PCDH15 contains an intragenic microsatellite marker that was associated with dyslipidaemia in a Finnish cohort, and later found to be associated with a single nucleotide polymorphism within *PCDH15* (Huertas-Vazquez *et al.*, 2010). Higher serum triglycerides and total cholesterol were identified in two mouse models of *Pcdh15* (Huertas-Vazquez et al., 2010). Hyperlipidaemia has not yet been confirmed in human subjects with DFNB23 or Usher syndrome 1F.

5 ***CDH3*** **causing macular dystrophy**

Variants in *CDH3* have been reported to underlie two syndromes: hypotrichosis with juvenile-onset macular dystrophy (HJMD; OMIM: 601553) and ectodermal dysplasia, ectrodactyly and macular dystrophy syndrome (EEMS; OMIM: 225280). The variable digital features of ectrodactyly, syndactyly, polydactyly most prominently distinguish EEMS from HJMD. Ectodermal dysplasia refers to developmental abnormalities of two or more ectodermally derived structures such as the skin, eccrine glands, teeth, mucous membranes, nails and hair.

CDH3 encodes P-cadherin (placental) which is expressed in various epithelial tissues including the retinal pigment epithelium, hair follicles, and mammary glands. *CDH3* was originally identified in developing ectodermally-derived epithelial cells in *C. elegans* (Pettitt *et al.*, 1996). Members of the cadherin superfamily were known to be important in epithelial morphogenesis in vertebrates. Loss-of-function mutations in *Cdh3* affected the morphogenesis of the tip of the nematode tail, suggesting compensatory mechanisms in other cells expressing Cdh3. Since two other cadherin-related genes had previously been identified in *C. elegans*, the gene was designated *Cdh3*.

5.1 Identification of *CDH3* variants in hypotrichosis with juvenile macular dystrophy

A familial association of macular dystrophy with hypotrichosis was first reported in 1935 (Wagner, 1935) followed decades later by other confirmatory reports (Johnston *et al.*, 1973, Kroll, 1981, Toussaint *et al.*, 1978, Marren *et al.*, 1992). Autosomal recessive inheritance was suggested in most reports as the most likely mode of inheritance. Clinical observations indicated that the RPE is primarily affected in this disorder.

Homozygosity mapping was undertaken in 4 consanguineous pedigrees with 11 affected individuals exhibiting congenital hypotrichosis and juvenile-onset macular dystrophy (HJMD: OMIM – 601553) from a Druze population in Northern Israel. This analysis localised the defective gene to the 16q22.1 locus (Sprecher *et al.*, 2001). *CDH3* was considered the most likely candidate gene within the locus, as it was expressed in both the retina and hair follicles. Sequencing of the *CDH3* coding region identified a homozygous deletion (981delG), within exon 8, resulting in premature termination of transcription 23 codons downstream. Reduced immunostaining for P-cadherin was demonstrated in skin biopsies from an affected individual (Sprecher et al., 2001).

5.2 *CDH3* gene structure and disease-associated variants

CDH3 has 5 extracellular cadherin repeat-like domains, a signal peptide, transmembrane and cytoplasmic domain; a classical cadherin (Figure 2). The majority of reported variants in *CDH3* are truncating and associated with HJMD (Figure 7, Supplementary Table 5). However, although HJMD and EEMS are identified generally as separate disorders, the observations that several variants may cause both syndromes, and the overlapping phenotypic features suggest that these disorders may be considered as a spectrum (Basel-Vanagaite *et al.*, 2010, Hull *et al.*, 2016). Hypotrichosis and macular dystrophy appear consistent, although limb malformations and other ectodermal manifestations may be present in EEMS. There are no clear criteria on which to predict a particular disease manifestation based on the identified *CDH3* variant. Variants affecting the *CDH3* calcium binding sites in the extracellular cadherin domains may have a detrimental effect on homodimerization within the RPE; for example the p.Arg503His variant associated with HJMD or the p.Asn322Ile variant associated with EEMS (Indelman *et al.*, 2002, Kjaer *et al.*, 2005).

The presence of disease modifiers relating so the systemic manifestations of the HJMD/EEMS spectrum is suggested by the report of a single individual with biallelic truncating variants in *CDH3* who exhibited bilateral macular dystrophy in the absence of frank hypotrichosis (Khan and Bolz, 2016). Furthermore, interfamilial and intrafamilial variation in limb abnormalities has been noted in EEMS (Basel-Vanagaite et al., 2010).

5.3 Clinical phenotype

5.3.1. Hypotrichosis with juvenile macular dystrophy (HJMD)

Sprecher *et al* summarise the key clinical findings across 4 pedigrees with HJMD (Sprecher et al., 2001). Typically, individuals are born with normal hair but develop alopecia around 3 months of age, with partial regrowth around the age of adolescence. Fusiform beading along the hair shaft on scanning electron microscopy is typical, with 180° twisting of the hair shaft – morphology consistent

with pili torti. Some growth of scalp hair may occur at adolescence although facial and body hair is unaffected. Eyelashes and eyebrows are unaffected, which together with the absence of digital abnormalities distinguishes the disorder from EEMS.

Individuals typically become symptomatic in the first two decades with symptoms of central visual disturbance related to macular dysfunction (Sprecher et al., 2001, Hull et al., 2016). More detailed retinal phenotyping from 10 individuals identified areas of marked, confluent chorioretinal atrophy at the posterior pole, contiguous with the optic disc in nine individuals. Fundus autofluorescence imaging reveals hypoautofluorescence in the areas of atrophy, indicating loss of the RPE, which may be surrounded by a hyperautofluorescent signal. In early disease, there may be diffuse hyperautofluorescence with mild disturbance of the ellipsoid band on OCT imaging. There is progressive thinning of the central retina. In later disease, outer retinal degeneration is seen with subretinal pigment clumps, retinal tubulations and macular edema (Hull et al., 2016).

Electroretinography may fall within normal limits for age in early disease. Most individuals demonstrate an abnormal P50 response on pattern ERG, consistent with the observed distribution of RPE loss. However, abnormalities in both dark-adapted and light-adapted responses suggests that retinal function may be affected more generally than may be predicted based on the distribution of disease seen on fundus autofluorescence. Macular dystrophy in HJMD appears to be progressive, although some patients may maintain vision over a long-period (Hull et al., 2016).

5.3.2 Ectodermal dysplasia, ectrodactyly and macular dystrophy syndrome (EEMS)

EEMS describes a syndrome of macular dystrophy with ectodermal dysplasia, which is characterised by hypotrichosis, sparse eyebrows and eyelashes and partial anodontia. Patients with EEMS may demonstrate digital malformations including ectrodactyly (the absence of one or more central digits of

the hand, with or without a central cleft), camptodactyly (permanent fixed deformity of the proximal phalanx), polydactyly of the feet (increased number of digits), and syndactyly (joining of one or more digits). Ectrodactyly and syndactyly are the most common limb defects (Kjaer et al., 2005). The hands appear to be more severely affected than the feet.

Hypotrichosis with syndactyly and retinal degeneration was first described in 1956, although the syndrome of EEMS was coined in 1983 in five individuals from a single consanguineous pedigree in a remote island in Japan (Ohdo *et al.*, 1983). The patients were characterised by macular dystrophy with central pigment mottling, without apparent peripheral retinal degeneration which separated it phenotypically from other described syndromes of ectodermal dysplasia without features in the ocular fundus. All affected individuals had sparse hair growth, including involvement of eyelashes and eyebrows, and variable digital features of ectrodactyly, syndactyly, and polydactyly.

In 2005, molecular genetic testing of the *CDH3* gene was undertaken in two pedigrees with EEMS, given the overlapping phenotypic features with HJMD, known to be caused by variants in *CDH3* (Kjaer et al., 2005). Mutation analysis identified homozygous variants in *CDH3* which segregated with individuals affected by EEMS. A role for *CDH3* in the development of the human hand was suggested with expression of CDH3 in the interdigital mesenchyme in mice embryos which was consistent with the EEMS phenotype (Kjaer et al., 2005).

The retinal phenotype in EEMS has not been studied in detail with multimodal retinal imaging or electroretinography. However, findings from colour fundus imaging suggest a broadly similar retinal phenotype to that observed in HJMD characterised by a pigmentary maculopathy with RPE loss and relative preservation of the peripheral retina and retinal vasculature.

6. Cadherins in the outer retina

6.1 - Photoreceptors

6.1.1. CDH23 and PCDH15

CDH23 and *PCDH15* are members of the cadherin related molecule subfamily. Despite their names, they are neither protocadherins nor classical cadherins. Biallelic variants in *CDH23* and *PCDH15* may cause Usher syndrome type 1D and 1F, respectively, as described above. CDH23 and PCDH15 engage in heterophilic trans interactions with each other. Unlike classical cadherins, this adhesion is not mediated by EC1/EC1 β-strand swapping, rather these proteins engage in a "handshake" of anti-parallel EC1/EC2 interactions (Sotomayor *et al.*, 2012). Both of these cadherin related molecules are expressed by sensory hair cells in the inner ear and by photoreceptors in the retina. In this section, we discuss their known interactions within the inner ear to hypothesize on their likely interactions and function within the retina.

Auditory and vestibular hair cells are so named for their apical hair bundles, tufts of stereocilia arranged in parallel rows of graduated length with a single primary cilium (or kinocilium) at the apex. It should be noted that the structure of hair cells is most closely aligned to microvilli than to cilia since an axoneme or microtubule is absent; the term "stereocilia" may therefore be considered to be a misnomer. The photoreceptor outer segment is a modified primary non-motile cilium of which the connecting cilium is a part (the "transition zone" of a conventional cilium). The kinocilia within the inner ear are true, specialised primary cilia. The stereocilia are connected by heterophilic interactions between PCDH15 and CDH23 at transient lateral links and tip-links (Siemens *et al.*, 2004, Kazmierczak *et al.*, 2007). These tip-links are essential for mechanotransduction, the process through which deflection of the hair bundle depolarizes the hair cell, signalling neurotransmitter release.

Mice with biallelic loss of function mutations in either *Pcdh15* or *Cdh23* are deaf with stereocilia dysmorphologies, suggesting that these cell adhesion molecules contribute to stereocilia development or maintenance in addition to mechanotransduction (Alagramam et al., 2001a, Di Palma et al., 2001a). Neuromast cells, part of the zebrafish lateral line mechanosensory system that detects water displacement, express *Pcdh15a* where the protein complexes with integrin α8 to regulate cilia biogenesis via activation of the small GTPase RhoA (Goodman and Zallocchi, 2017).

Further work was undertaken to characterise the role of cdh23 in zebrafish brain and retinal tissue (Glover *et al.*, 2012). Expression of cdh23 was identified in nuclei within the central nervous system and in a small subset of GABAergic amacrine cells, but not in photoreceptors. Moreover, larvae homozygous for deleterious cdh23 variants did not exhibit morphological retinal degeneration or functional deficits (Glover et al., 2012). These observations suggest different roles of cdh23 within the retina in humans. Indeed, single-cell RNA-Seq analysis identified the strongest expression of CDH23 in human retinal tissue within Müller-glia cells (NCBI) although expression was also identified in rod photoreceptors (Supplementary Figure 1). Mice lacking the Cdh23 gene demonstrated impaired auditory cortex connectivity or interneuronal development of cdh23 expressing interneuron precursors with scattered localisation, disordered polarity and auditory seizures (Libé-Philippot *et al.*, 2017). This striking finding suggests a direct role for cdh23 in CNS neuronal development within the auditory cortex beyond isolated dysfunction of the cochlea associated with variants in cdh23.

Of the 12 *Cdh23* murine models which harbour functional null alleles, none exhibit severe retinal degeneration as seen in human USH1D patients with biallelic nullizygous variants in *CDH23*, although they do exhibit waltzer phenotypes of severe hearing and vestibular impairment seen in DFNB12 patients (Di Palma et al., 2001a, Wada *et al.*, 2001, Wilson *et al.*, 2001, Holme and Steel,

2002). This finding is consistent with other USH1 knockout mouse models (USH1B, 1C, 1F and 1G). A detailed investigation of the retinal phenotype in two Ames waltzer mice (*Pcdh15*$^{av\text{-}5J}$ and *Pcdh15*$^{av\text{-}Jfb}$) with full field electroretinography demonstrated that although mice homozygous for these mutant Pcdh15 alleles had profound early-onset hearing loss, there was only a modest reduction in a- and b-wave ERG amplitudes when compared with heterozygous controls (Haywood-Watson *et al.*, 2006).

Both *Pcdh15* and *Cdh23* genes produce transcriptional variants through alternate start sites or alternative splicing. Reiners *et al* were able localise Pcdh15 and Cdh23 in the mouse retina by immunohistochemistry (Reiners *et al.*, 2005). Specifically, Pcdh15 was localised to OS of both rod and cone photoreceptors as demonstrated through co-localisation with lectin/peanut agglutinin, with immunogold labelling of Pcdh15 confirming OS localisation on immunoelectron microscopy (Reiners et al., 2005) Furthermore, Cdh23 expression was seen within the IS in the murine retina (Reiners et al., 2005). A further study could not identify full-length Cdh23 in the murine retina, although identified a shortened form without any extracellular EC repeats, and by immunoelectron microscopy found Cdh23 localized to the basal body of the mouse IS (Lagziel *et al.*, 2009). A separate study identified very low levels of Pcdh15 localized to the IS but was unable to detect Cdh23 in mouse retina (Sahly *et al.*, 2012). Immunodetection of Cdh23 in the murine retina may be heavily dependent on experimental methods, and complicated by transcriptional variants. Alternatively-spliced CDH23 isoforms (Di Palma *et al.*, 2001b, Lagziel et al., 2009) may explain the absence of *CDH23* in rod and cone photoreceptors in a single-cell RNA sequencing study of *ex vivo* and iPSC-derived retinal organoids (Cowan *et al.*, 2020).

In macaque and human photoreceptors, both PCDH15 and CDH23 were localized to the calyceal processes, actin-rich finger-like protrusions from the apical IS that form a collar around the

basolateral OS (Sahly et al., 2012). Interestingly, calyceal processes are a feature of vertebrate photoreceptors (i.e. primates, mammals, amphibians etc.) although rodents lack these structures. Experiments using *Xenopus tropicalis* localised PCDH15 to links between calyceal processes and OS in rods and cones by immunogold labelling on electron microscopy (Figure 3C) (Schietroma *et al.*, 2017). Morpholino knockdown of either *Pcdh15* or *Cdh23* in *Xenopus tropicalis* resulted in fewer calyceal processes per photoreceptor and dysmorphic rod and cone OS, with a reduced ratio of OS to cell nuclei in Cdh23 morphant retinas. These abnormalities included shorter and wider OS with an occasional mass of rod disc overgrowth outside of the expected cylindrical shape, suggesting that the calyceal processes function to constrain the shape of the OS (Schietroma et al., 2017). The spacing between calyceal processes in the retina appear very similar to the stereocilia, suggesting that CDH23-PCDH15 heterodimers may form in the retina at the base of the OS between the calyceal processes. It has been further proposed that the calyceal processes of the photoreceptor are the morphological homologue of the stereocilia of hair cells in the inner ear where other USH1 proteins are expressed (Sahly et al., 2012). One attractive explanation is that PCDH15/CDH23 heterophilic links bind the calyceal processes to the OS, and potentially to each other, to limit the lateral expansion of newly forming rod discs and guide the tapering of the cone OS.(Sahly et al., 2012, Schietroma et al., 2017) It would seem that these constraints are required in amphibian and primate retina, but that mouse photoreceptors use alternative mechanisms.

In order to further evaluate the mechanisms behind the apparent absence of retinal degeneration in *Pcdh15*$^{-/-}$ mice, zebrafish were shown to harbour two closely related *Pcdh15* genes which selectively affect ocular and auditory function following knockdown with inhibitory morpholino oligonucleotides. Specifically, OS retinal morphology, alignment and RPE interdigitation was affected following inhibition of the retinal *Pcdh15* gene (Seiler *et al.*, 2005). The intriguing

observation of duplicated genes that are expressed in different organelles has not yet been demonstrated in other species.

Pcdh15 appears to serve a role in the embryogenesis of numerous neuronal (inner ear, eye) and non-neuronal (kidney, spleen, gut) tissues. In situ hybridization identified the uniform expression of *Pcdh15* across the brain in the wildtype mouse embryo at day 12 (Murcia and Woychik, 2001). By embryonic day 18, expression of both *Pcdh15* and *Cdh23* was found specifically in interneurons that had migrated to within the auditory cortex. In mice mutant for either adhesion molecule, a subset of interneurons destined for the auditory cortex failed to migrate out from the medial ganglionic eminence, a phenotype likely related to defects in cell polarity (Libé-Philippot et al., 2017). The expression of Pcdh15 in microvilli-containing cells in the embryo, inner ear, retina and other tissues suggests a specialised role in the function of these structures in a variety of tissues. Further work is required to characterise the roles of Pcdh15 in brain and non-neuronal tissues.

Other Usher syndrome related proteins complex with PCDH15 and CDH23 (Figure 2). Harmonin (USH1C) is an alternatively spliced scaffolding molecule with two or three PDZ domains. CDH23 has a C-terminal PDZ-interacting motif through which it binds to PDZ2 of harmonin (Siemens *et al.*, 2002, Boëda *et al.*, 2002). SANS (USH1G) is a scaffolding protein composed of ankyrin repeats, a central domain, a SAM domain, and a C-terminal PDZ-interacting motif. SANS and harmonin form a highly stable interaction involving the N-terminal domain and PDZ1 of harmonin and the SAM domain and PDZ-binding motif of SANS (Yan *et al.*, 2010). Finally, MYO7A (USH1B) binds to the SANS central domain and links the entire complex to the actin cytoskeleton. However, it should be noted that harmonin may interact directly with actin filaments without direct involvement of MYO7A (Boëda et al., 2002), and CDH23 can form a ternary complex directly with MYO7A and harmonin, further supporting the stability of these complexes (Bahloul *et al.*, 2010). At the other end of the

complex, PCDH15 can bind the long form of whirlin (USH2D), a scaffolding molecule homologous to harmonin (Michel *et al.*, 2020). Moreover, PCDH15 also binds via its c-terminal PDZ-binding motif (PBM) to the second PZD domain of harmonin directly (Reiners et al., 2005). Since these molecules are all present in rod outer segments, it is possible that these proteins interact directly there, although there is as yet no direct evidence of this. PCDH15 can also bind directly to MYO7A, suggesting that a similar protein complex links PCDH15 to the actin cytoskeleton as CDH23 (Senften *et al.*, 2006). The other USH1 proteins co-localize with PCDH15/CDH23 in calyceal processes in primate retina (Sahly et al., 2012). While they may serve additional functions in the retina (e.g., MYO7A facilitates transport through the connecting cilium), it is likely that their contribution to preventing retinal degeneration is centered on their core function linking points of PCDH15/CDH23 adhesion to actin filaments or cytoskeleton in calyceal processes (Schietroma et al., 2017). In addition, the two USH1 cadherins are also expressed in the outer plexiform layer at the photoreceptor synapses,(Reiners et al., 2006, Lagziel et al., 2009) and both CDHR1 and PCDH15 expression has also been identified in retinal ganglion cells (Reiners et al., 2005, Berg *et al.*, 2019). Their roles in these cell types are not well characterised.

Digenic inheritance of deafness has been reported for compound heterozygous variants in *Cdh23* and *Pcdh15* in mice which were not present in single gene heterozygous controls (Zheng *et al.*, 2005). In humans, three unrelated families demonstrated digenic inheritance of USH1 with compound heterozygous truncating variants in *CDH23* and *PCDH15* (Zheng et al., 2005). This observation further validates the hypothesis that the arrangement of USH1 proteins in the retina may parallel the USH1 proteins networks characterised in the inner ear. Moreover, these data further support a direct interaction between CDH23 and PCDH15 in the human retina since digenic inheritance was found to underlie USH1 rather than recessive non-syndromic deafness (Zheng et al., 2005). Furthermore,

digenic inheritance of *CDH23* and *PCDH15* provides an additional mechanism to explain the observation of clinically indistinguishable phenotypes between different forms of USH1.

6.1.2. CDHR1

CDHR1 (cadherin related family member 1) was discovered in a screen for genes expressed specifically in the retina and was initially named prCAD for photoreceptor cadherin as its expression is most prominent in photoreceptors (Rattner *et al.*, 2001, Burgoyne et al., 2015) and was also referred to as *PCDH21* in the initial clinical reports (Bolz et al., 2005, Henderson et al., 2010, Ostergaard et al., 2010). Since the dominant nomenclature of the gene is CDHR1 in clinical practice, genetic testing, and the recent literature, we use the term to refer to the gene and its murine homolog *(Cdhr1)* throughout this review.

Targeted disruption of *Cdhr1* in the mouse germline proved helpful in further elucidating the function of the protein (Rattner et al., 2001). Histological analysis of *Cdhr1*$^{-/-}$ mice at 6 months of age demonstrated loss of photoreceptor cells. OS proteins (i.e. rhodopsin) otherwise appeared correctly localised suggesting that Cdhr1 does not function in the trafficking of OS proteins, nor is this a likely mechanism of photoreceptor cell death in *Cdhr1*$^{-/-}$ mice (Rattner et al., 2001). Electroretinography in *Cdhr1*$^{-/-}$ mice at 2 months of age identified a 2-3 fold reduction in scotopic responses compared to wildtype and a 2-fold reduction in photopic b-wave saturating amplitude (Rattner et al., 2001). Longitudinal deep phenotyping of the *Cdhr1*$^{-/-}$ mouse model showed progressive outer retinal degeneration using OCT imaging with poorly formed OS and loss of the ellipsoid zone after 3 months of age(Yusuf *et al.*, 2021). Electroretinography showed functional deficits affecting both rods and cones out of proportion to outer nuclear layer thinning at 1-month(Yusuf et al., 2021), which may be

explained by shortened photoreceptor OS. These data indicate that *CDHR1* is important in normal rod and cone photoreceptor function.

CDHR1 is unique as the only cadherin currently described wherein biallelic variants result in apparent non-syndromic retinal degeneration. Immunoelectron microscopy identified CDHR1 in the OS at the leading edges of plasma membrane evaginations with physical connections to the periciliary ridge of the IS (Figure 3C) (Burgoyne et al., 2015). The antibody used in these experiments was targeted against the C-terminus which is located intracellularly within the photoreceptor OS discs, leaving the connections formed from CDHR1 ectodomains unstained. These junctions were only present in immature discs at the base of the OS, suggesting that these connections may provide an external scaffold to stabilise or control the process of evagination and/or horizontal disc elongation crucial for OS disc assembly (Burgoyne et al., 2015). Consistent with this, photoreceptors in *Cdhr1*$^{-/-}$ mutant mice had shortened, misaligned OS (Rattner et al., 2001). These observations are consistent with findings on light and electron microscopy, as well as with OCT imaging in *Cdhr1*$^{-/-}$ mice(Yusuf et al., 2021) .

Rattner *et al* demonstrated that antibodies directed against the N-terminus or C-terminus of CDHR1 did not identify CDHR1 in mature OS discs, a finding later confirmed independently (Burgoyne et al., 2015, Rattner et al., 2001). The restriction of CDHR1 expression to the immature or nascent disc edges, and the mechanism by which the physical separation of CDHR1-based connections between the inner and OS are lost may be explained by the proteolytic cleavage of the CDHR1 ectodomain, whose significance was not understood at the time of its identification (Rattner *et al.*, 2004). Full length CDHR1 has a molecular weight of approximately 120kDa, although immunoblot analysis using N-terminal and C-terminal antibodies identified a 95kDa N-terminal soluble fragment and a 25kDa C-terminal fragment associated with the OS. This cleavage occurs in the 6th cadherin repeat close to the transmembrane domain (between p.Thr669 and p.Thr670 in mice) within a highly conserved region

(Rattner et al., 2004). This catalytic event may release CDHR1-mediated connections, allowing OS discs to grow out towards the RPE. Further, this may explain why CDHR1 is no longer detectable in mature discs as the N-terminal fragment disperses after cleavage, and the C-terminal fragment is degraded by the intracellular proteolytic pathways as the OS discs mature.

It was recently suggested that ADAM10 (disintegrin and metalloproteinase family member 10), an alpha-secretase enzyme that forms part of the ADAM family that have known roles in cell partitioning during development, may be a candidate for the enzyme responsible for the cleavage of the CDHR1 ectodomain (Cisneros *et al.*, 2020). ADAM10 cleaves the ectodomains of a number of cadherins and protocadherins, and is co-expressed with a number of retinal cadherins in the developing chicken retina (Yan *et al.*, 2011). An increased proportion of cleaved, C-terminal CDHR1 fragments were identified in *Sfrp1*$^{-/-}$ (Secreted Frizzled Related Protein 1) mice when compared to wildtype and rd10 mice (Cisneros et al., 2020). As SFRP1 can act as a negative regulator of ADAM10 (Esteve *et al.*, 2011), this suggests that CDHR1 could be a substrate for ADAM10. Indeed, *Sfrp1*$^{-/-}$ mice demonstrate mild OS morphological alterations and with reduced rod and cone responses compared to age-matched wildtype mice at 9-months of age. It was suggested that this degeneration may occur through altered processing of other cadherins and protocadherins such as CDHR1, which is consistent with the similar, but milder phenotype than that seen in *Cdhr1*$^{-/-}$ mice. Further studies are required to directly link ADAM10 with the proteolytic processing of the CDHR1 ectodomain; co-immunoprecipitation studies or co-expression in cell culture may be indicative. Moreover, it is unclear how the cleavage of the CDHR1 ectodomain is timed to coincide with the maturation of the OS discs, before which the physical links between inner and OS appear necessary in disc morphogenesis. If proven, *ADAM10* and *SFRP1* may be candidate genes in unsolved cases of cone or cone-rod dystrophies.

CDHR1 appears to interact directly, both *in vitro* and *in vivo* with PROM1. Biallelic null variants in PROM1 result in pan-retinal dystrophy and heterozygous variants (most commonly p.Arg373Cys) result in predominant cone involvement affecting the macula (Cehajic-Kapetanovic *et al.*, 2019). The overlapping clinical phenotypes in CDHR1 and PROM1-related cone-rod and macular dystrophies align to the localisation of PROM1 in nascent disc membranes in rod OS. It is possible that degeneration occurs in these two disorders through an overlapping molecular mechanism affecting disc morphogenesis. However, PROM1 is expressed throughout cone OS. Mutant Prom1 (Arg373Cys) was found to co-localise with full-length CDHR1 on immunohistochemistry and co-immunoprecipitation studies (Yang *et al.*, 2008). Proteolytic processing of CDHR1 was found to be partially reduced in *Prom1* mutant mice, suggesting that the interactions of PROM1 and ADAM10 are worthy of further investigation. It is possible that disordered OS disc morphogenesis in the PROM1 mutant mouse may affect the expression, localisation and/or function of ADAM10 (i.e. by an indirect effect). Prom1 has been shown to interact with actin filaments and may provide a mechanism through which CDHR1 may interact with the actin cytoskeleton within photoreceptor OS (Yang et al., 2008). PROM1 co-localised with full length rather than truncated CDHR1 (Yang et al., 2008), suggesting that the proteins are no longer coupled following cleavage of the CDHR1 ectodomain. However, further work is required to determine the function of the interaction between the two proteins in photoreceptor OS disc formation and their interaction with associated proteins such as ADAM10 and SFRP1.

Although the role of CDHR1 in the mature retina is well characterised, its role in photoreceptor development is less clear. During retinal development, it has been shown that CDHR1 localises to the site of future OS development prior to other proteins expressed in the OS (i.e. rhodopsin) (Rattner et al., 2001). This finding supports the essential function of CDHR1 in OS disc assembly, which is necessary to later house the phototransduction proteins. A relatively consistent expression of CDHR1 in murine retina has been demonstrated beyond 2 weeks post-natal age, although CDHR1 may be

detected in whole eye tissue as early as 12 days post-conception in embryos which increases through gestation (Fu *et al.*, 2018). An intriguing observation is that cone function appears to improve on light-adapted electroretinography in *Cdhr1*$^{-/-}$ mice to 6 months followed by progressive decline at rate similar to rod function (Yusuf et al., 2021). Further investigation analysing temporal expression of Cdhr1 in rods and cones in wildtype mice may shed light on the underlying mechanism behind this observation.

6.1.3. Classical cadherins

In the instances discussed above, cadherin superfamily members mediate adhesion between compartments of the same cell. The more traditional understanding of cadherin-mediated adhesion is that between cells. The outer limiting membrane (OLM) separates photoreceptor cell bodies from the IS and is made of heterotypic junctions between Müller glia and photoreceptors. These specialized junctions include the classical cadherin CDH2 (formerly N-cadherin or neural cadherin)(Matsunaga *et al.*, 1988, Koike *et al.*, 2005, Paffenholz *et al.*, 1999, Campbell *et al.*, 2006) and other traditional components of adherens junctions (e.g., α- and β-catenin) as well as proteins more typically associated with tight junctions (e.g., claudins, JAM adhesion molecules) (Omri *et al.*, 2010). This is consistent with the dual role of the OLM maintaining retinal structure and serving as a barrier. The retinal phenotype of patients with CDH2 mutations has not been reported, although it has been reported in patients with Peter's anomaly (Reis *et al.*, 2020).

6.2 - Retinal pigment epithelium

The RPE is an epithelium, and as such, its structural integrity is maintained in part by adherens junctions. However, unlike most epithelia which rely on CDH1 to maintain adherens junctions, the cells of the RPE predominately express *CDH3* (Figure 3A) (Yang et al., 2018). There has been some controversy around this point in the literature. In mouse, the developing RPE was found to express

Cdh2 at embryonic day (E) 10.5, switching to *Cdh3* from E12 (Xu *et al.*, 2002). It is unknown if this switch also occurs in humans, but primary human RPE cells form an orderly epithelium while predominantly expressing *CDH2* in culture (Kaida *et al.*, 2000, McKay *et al.*, 1997, Yang et al., 2018). A recent study quantitatively measured mRNA for *CDH1*, *CDH2*, and *CDH3* in human and mouse RPE, finding that *CDH3* made up ~80-90% of the total of the three, while confirming the predominance of *CDH2* in cultures (Yang et al., 2018). This, together with the functional consequences of *CDH3* mutation in humans discussed below, confirms that CDH3 is the major classical cadherin of the RPE. Deficiency of *CDH1* and *CDH2* are embryonically lethal in mice suggesting a critical function in non-ocular tissues (Radice *et al.*, 1997). However, it is possible that hypomorphic variants in these cadherins expressed in the RPE may be viable and result in RPE dysfunction, although this has not yet been described.

The RPE extends apical processes around the photoreceptor OS. This close association supports visual function by cycling all-trans retinal to 11-cis retinal and by continually phagocytosing distal membrane discs released by photoreceptors. Cadherin superfamily members have not been described at these contacts, which rather involve αvβ5-integrin and neural cell adhesion molecule (NCAM; CD56) (Nandrot *et al.*, 2006).

7. Cadherins in the inner retina

The neural retina is a complex extension of the central nervous system composed of over 100 types of neurons in mouse (Yan *et al.*, 2020a, Shekhar *et al.*, 2016, Rheaume *et al.*, 2018) and over 50 types in human and other primates (Yan *et al.*, 2020b, Peng *et al.*, 2019). This cellular complexity is mirrored by diverse and overlapping expression of many members of the cadherin superfamily. Numerous studies have demonstrated cell-type-specific expression of classical cadherins,

protocadherins, and atypical cadherins in the vertebrate inner retina, with roles in neural circuit formation and function (Etzrodt *et al.*, 2009, Honjo *et al.*, 2000, Wohrn *et al.*, 1998, Matsunaga *et al.*, 2014). However, no human disease associations have yet been established with cadherins expressed in the inner retina.

7.1. Classical cadherins

Cdh2 and *Cdh4* (R-cadherin) are expressed early and broadly in the developing retina and are important for establishing its basic organization. *Cdh4* knockdown by morpholino in zebrafish resulted in failures of retinal lamination, excessive neuronal apoptosis, and small eyes (Babb *et al.*, 2005). *Cdh2* mutation in zebrafish initiated a host of retinal malformations, including disruptions in layer formation and patterning, retinal ganglion cell (RGC) axon pathfinding defects, and invasion of retinal neurons into the brain (Masai *et al.*, 2003, Malicki *et al.*, 2003). These mutants also exhibited coloboma as the choroid fissure failed to close. Consistent with this, targeted disruption of α-catenin in the developing mouse eye induced a failure in optic fissure closure and coloboma (Chen *et al.*, 2012). These phenotypes are consistent with the fundamental role of cadherin adhesion at adherens junctions to facilitate cell migration and tissue integrity.

As neuronal subtypes in the inner retina each stratify their processes in specific sublayers within the inner plexiform layer (IPL, Figure 3A), cell-type-specific expression is often revealed by layer-specific localization. This has led to the hypothesis that cadherin superfamily members contribute to an adhesion code of target recognition molecules by facilitating interaction between synaptic partners. The clearest example of this came from a pair of studies focused on type II classical cadherins in mouse retina. The OFF bipolar cell type BC2 were found to express *Cdh8*, while the ON bipolar cell type BC5 expressed *Cdh9*. The cadherins were necessary and instructive for the laminar targeting of these bipolar cells' axons within the IPL where they form synapses onto ON-OFF

direction selective ganglion cells (ooDSGCs), and starburst amacrine cells (SACs): Loss of function resulted in disorganized lamination, while ectopic expression redirected stratification (Duan *et al.*, 2014). There are four types of ooDSGCs in mouse corresponding to the four cardinal directions. Those selectively responsive to ventral or dorsal motion (V-ooDSGCs and D-ooDSGCs) depended on a combination of CDH6, CDH9, and CDH10 for their normal bistratified dendritic colocalization with SAC dendrites, while nasal-preferring N-ooDSGCs required CDH7 (Duan *et al.*, 2018). Type II cadherins can have homophilic and heterophilic ligands, and SACs express both *Cdh6* and *Cdh18* (encodes ligand for CDH7). SAC-specific deletion of *Cdh6* or *Cdh18* resulted in mistargeted ooDSGC dendrites, but none of the cadherin mutants had inappropriate SAC targeting (Duan et al., 2014, Duan et al., 2018). SACs stratify their dendrites to specific sub-laminae of the IPL in the early postnatal period and provide a scaffold of type II cadherins to direct bipolar cell axons and ooDSGC dendrites to their proper targets (Figure 3B).

Classical cadherins also facilitate targeting of RGC axons to their synaptic partners in the brain. In chick, *Cdh2*, *Cdh4*, and *Cdh13* (T-cadherin) are expressed in distinct layers of the optic tectum, the central target of RGC axons, and *Cdh2* is required for proper laminar organization of RGC axons (Inoue and Sanes, 1997, Miskevich *et al.*, 1998). In mouse, *Cdh6* and *Cdh3* are expressed by a RGC type involved in a non-image-forming circuit, and CDH6 is required for the proper targeting of their axons to the olivary pretectal nucleus (Osterhout *et al.*, 2011, Garrett *et al.*, 2018). There is also evidence that both CDH6 and CDH3 serve a yet undefined adhesive role in these neurons within the retina (Garrett et al., 2018).

7.2. Protocadherins

The non-clustered δ-Pcdhs have individual expression patterns in the retina consistent with cell-type-specific expression and could contribute to the adhesion code for proper neural wiring in the retina,

although functional studies to test this have not been completed (Etzrodt et al., 2009). *Pcdh7* (NF-pcdh) is important for RGC axon outgrowth and guidance in *Xenopus* (Leung et al., 2015, Piper et al., 2008), and in zebrafish, individual δ-Pcdhs are expressed in columns of neurons in the optic tectum (Cooper *et al.*, 2015). These could be involved in biasing synaptic connections, contributing to an adhesion code for central projections.

The clustered protocadherins (cPcdhs) are expressed throughout the retina (Lefebvre et al., 2008, Ing-Esteves et al., 2018). Most functional analyses have focused on the γ-Pcdhs, 22 homophilic protocadherin isoforms expressed from the *Pcdhg* locus, each with a distinct extracellular domain but all with a common C-terminal cytoplasmic domain. Immunolabeling for the constant domain revealed punctate localization throughout the synaptic IPL and OPL (outer plexiform layer), but no clear pattern for specific isoforms (Wang *et al.*, 2002). Mice without the entire cluster died at birth with severe apoptosis in the spinal cord (Wang et al., 2002). When mutation was limited to the retina, the dominant phenotype was also excessive neuronal cell death (Lefebvre *et al.*, 2008). This appears to be an exacerbation of normal developmental apoptosis, as cell death in the retina and elsewhere can be prevented by loss of function mutation in *Bax,* a pro-apoptotic BCL-2 family member (Lefebvre et al., 2008, Weiner *et al.*, 2005, Prasad *et al.*, 2008). Further, not all cell types undergo excessive apoptosis in *Pcdhg* mutants, but those that do also persist in excess in *Bax* mutants (Prasad et al., 2008, Lefebvre et al., 2008). α- and β-Pcdhs likely also contribute in some fashion: deletion of the *Pcdha* or *Pcdhb* (alone or together) had no effect on neuronal survival, but the triple deletion (*Pcdha*, *Pcdhb*, and *Pcdhg*) resulted in more apoptosis than *Pcdhg* deletion alone (Ing-Esteves *et al.*, 2018). In a recent study, the role of γ-Pcdh isoform diversity was probed by generating a new allelic series of mouse mutants through simultaneous CRISPR/Cas9 targeting of all 22 variable exons. One key finding from this study was that only one isoform (γC4) was necessary or sufficient for neuronal survival: mice lacking all isoforms except γC4 survived with normal cell

number in the retina, while those lacking only γC4 died at birth with excessive apoptosis (Garrett *et al.*, 2019). There must be interactions specific to this isoform, either through unique protein sequence or unique localization, that are required to promote neuronal survival (Figure 2). This isoform is also the only γ-Pcdh under constraint in humans, suggesting its essential role in neuronal survival is conserved (Karczewski *et al.*, 2020, Garrett et al., 2019). This also likely explains why no γ-Pcdh retinopathies have been described in humans, as the consequences of loss of function in γC4 are widespread and incompatible with life.

The secondary phenotype in *Pcdhg* mouse mutants was a failure of self-avoidance in SACs. Like most amacrine and ganglion cell types, SACs are non-randomly distributed through the horizontal plane of the retina, forming mosaics of evenly spaced cell bodies with overlapping dendritic arbors. The amount of overlap varies between cell types, but for SACs the coverage factor is ~35, meaning any given SAC shares a domain with ~35 others (Keeley *et al.*, 2007). In retinas without the entire *Pcdhg* cluster, individual SACs failed to achieve their normal "starburst" morphology as dendrites became entangled with each other (Lefebvre *et al.*, 2012). The current hypothesis is that homophilic γ-Pcdh-mediated contacts trigger a repulsive signal to prevent sister dendrites from the same cell tangling with each other (Figure 3B). Stochastic isoform expression is unlikely to match between neighboring neurons, allowing neurites to distinguish between "self" and "non-self" and overlap with neighbors. Consistent with this, structural studies have suggested a mechanism by which cPcdhs form a lattice of dimers across membranes with matching isoforms, but only small complexes with even a single isoform mismatch between membranes (Rubinstein *et al.*, 2015, Brasch *et al.*, 2019). As in the cell death phenotype, only γ-Pcdhs are required for self-avoidance in SACs, but α- and β-Pcdhs are also likely involved, as the triple mutants have a more severe phenotype (Ing-Esteves et al., 2018).

7.3. Atypical cadherins

Calsyntenins (CLSTN1, CLSTN2, CLSTN3) are atypical cadherins with two EC repeats. All three are expressed in the developing retina with strongest and most sustained expression in the RGCs (de Ramon Francas *et al.*, 2017). *Cstn1* (also called *Alcα*) mouse mutants underwent progressive RGC death beginning at 3 months of age: Other retinal layers were unaffected, and intraocular pressure was not elevated (Nakano *et al.*, 2020). The mechanism for this cell death is unknown, but calsyntenins act as linkers between kinesin 1 motor proteins and vesicular cargo in microtubule transport (Konecna *et al.*, 2006), suggesting that deficiencies in transport along the ganglion cell axon could be the underlying cause.

At the other extreme of the cadherin related molecule subgroup, Fats (FAT1, FAT2, FAT3) have 34 EC repeats. In mouse, *Fat3* was found expressed in the IPL starting from the late embryonic period, where it was important for the normal development of amacrine cells. In null mutants, excess GABAergic amacrine cells migrated into the retinal ganglion layer, and more strikingly, some amacrine cells within the inner nuclear layer (INL) projected dendrites in the wrong direction towards the outer retina, making an ectopic plexiform layer within the INL (Deans *et al.*, 2011). This disorganization is consistent with the classic role of the Fat/Dachsous system in establishing planar cell polarity, first explored in *Drosophila*. Indeed, FAT3 in the IPL drives localization of Ena/VASP to the IPL, proteins that regulate actin filament dynamics and membrane protrusions, and mislocalization of Ena/VASP in the presence of FAT3 resulted in similar ectopic projections, suggesting that these phenotypes resulted from a failure of polarization (Krol *et al.*, 2016).

8. Retinal cadherins: uncertainties

Although considerable progress has been made in the identification and characterisation of retinal cadherins over the past 20 years, including the identification of four cadherins underlying monogenic

retinal degeneration, uncertainties remain regarding the roles and interactions of cadherins expressed in the retina. It is likely that some retinal cadherins have not yet been identified; genes encoding these proteins and their interacting partners may be candidates for unsolved cases of retinal degeneration or dysfunction. Fundamental questions remain regarding the roles of the four identified retinal cadherins underlying monogenic retinal degeneration and further work is required to elucidate their interacting partners, protein networks and function in the retina. Since cadherins contribute to diverse functions including retinal development, cellular adhesion and repulsion, OS development and structural maintenance, and form tight junctions between RPE cells, further elucidation of retinal cadherins may yet present therapeutic opportunities beyond inherited retinal degeneration.

Research into the roles of retinal cadherins is hampered by differences in retinal expression and function between species, the presence of splice isoforms (CDH23 and PCDH15), and variability of expression depending on age. However, inter-species differences in expression and function of retinal cadherins may yield important insights into their roles and redundancies (i.e. the expression of USH1 proteins in calyceal processes, absent from rodent photoreceptors). In this section, we consider the uncertainties relevant to the key cadherins identified as causes of monogenic retinal degeneration.

In the decade following the identification of *CDHR1* as a cause of human disease (2001), the main retinal phenotypes have been identified as cone-rod dystrophy - or rarely, retinitis pigmentosa - in individuals with biallelic null variants, and late-onset macular dystrophy in individuals with one or more hypomorphic variants. The spectrum of retinal phenotypes in late-onset macular dystrophy and their association with the increasing number of recognised hypomorphic sequence variants require further characterisation. Protein modelling based on disease-associated variants may yield additional perspectives on the key functional domains of CDHR1 by comparing missense variants that result in

cone-rod versus macular phenotypes. The prediction of peripheral retinal degeneration from the combination of variants is of interest since this relevant to inclusion in clinical trials. Furthermore, it is unclear why some individuals develop retinitis pigmentosa with hypomorphic alleles (Bessette et al., 2017), that may be more commonly associated with late-onset macular dystrophy. As yet unidentified disease modifiers are likely to play a role in the retinal phenotype. However, retinal phenotypes in patients with variants in the other monogenic retinal cadherinopathies appear more predictable with *CDH23* and *PCDH15* variants resulting in RP and *CDH3* with macular dystrophy. Hypomorphic CDHR1 variants may lead to mild functional deficits that are compensated for in the peripheral retina, perhaps by Prom1 (Carr *et al.*, 2021). However, further investigation is required to explore the mechanisms through which the higher order organisation of the outer segment is regulated, and whether this varies topographically according to retinal location. Accordingly, it is unclear as to why biallelic *CDH3* variants result in macular disease with the peripheral RPE appearing relatively unaffected.

Despite the elucidation of the clinical manifestations of *CDHR1*-associated retinal degeneration, the molecular and cellular mechanisms underlying them are less well understood. *CDHR1*-based contacts between the nascent/evaginating OS discs in rods and cones and the periciliary ridge of the IS has been characterised by immunoelectron microscopy as previously described (Burgoyne et al., 2015). However, it is unclear as to how the Cdhr1-based contacts allow the OS discs to mature to reach full length. Two hypotheses have been suggested: either the links are fluid within the IS and move along the ridge whilst remaining connected to the OS disc, or the *CDHR1* ectodomains are cleaved by an extracellular protease, and replaced by ongoing expression of *CDHR1* within the OS allowing evaginating, nascent horizontal discs to “walk” along the periciliary ridge of the IS. Direct experimental proof is necessary to confirm that ADAM10 cleaves the CDHR1 ectodomain, as has been shown with other retinal cadherins (Paudel *et al.*, 2013). In the latter model, further

characterisation of the activity of ADAM10 may be instructive. If ADAM10 can be shown to co-localise with CDHR1 in the most nascent OS discs, this would support the second hypothesis of CDHR1 expression, proteolysis and replacement in the process of disc morphogenesis. Identification of the binding partner of CDHR1 in the IS is also important to further delineate the process by which physical CDHR1-based links help to form OS discs. Horizontally aligned actin filaments or cytoskeleton within the periciliary ridge IS may well provide a scaffold to support horizontal OS disc outgrowth.

Elucidating the physiological roles of CDHR1 may help in further understanding of how pathological sequence variants result in photoreceptor cell death. Furthermore, in late-onset macular dystrophy, characterising the role of Cdhr1 may explain macular involvement with hypomorphic variants; a phenomenon seen with other genes (i.e. *PRPH2*). The mapping of degenerative pathways may suggest therapeutic opportunities for intervention. Moreover, they may suggest other candidate genes for retinal degeneration (such as ADAM10, and its regulators such as SFRP1) (Cisneros et al., 2020). *Sfrp1*$^{-/-}$ mice have been shown to have disorganised OS which is thought in part due to increased proteolysis of *CDHR1* through potentiation of the activity of ADAM10 (Cisneros et al., 2020). Further work is required to confirm these interactions, which if confirmed, may present the possibility of digenic inheritance in unsolved cases of retinal degeneration (as identified similarly with *ROM1* and *PRPH2*).

A key objective in the further characterisation of all USH1 subtypes is to map the network of interactions between the USH1 proteins within the retina. The expression of all USH1 proteins within the murine inner ear has led to a detailed description of the interactions of USH1 proteins with each other, and with the cytoskeleton (Figure 2). Within the retina, the USH1 proteins may interact analogously as previously described with the calyceal processes sharing structural similarities with

the stereocilia in the inner ear. As suggested by the indistinguishable clinical phenotype amongst USH1 patients, the USH1 proteins appear to act on the same network of structures. Further characterisation of the USH1 network may help to add to the understanding of the effect of missense variants within the relevant genes, the function of key interacting domains and possibly suggest therapeutic approaches that are independent of the affected gene. The USH1 network characterised within the inner ear will enable hypothesis-based research of the function of the calyceal processes, and how USH1 proteins interact to regulate rod photoreceptor OS disc development. Furthermore, a clearer understanding of the USH1 protein interactions within the retina may reveal the identity of as yet unidentified genes associated with USH1 (i.e. USH1E, 1H, 1K).

Müller glial cells are widely present in the vertebrate retina. Single-cell RNA-Seq data using human retinal tissue indicates high levels of Cdh23 expression in Müller glial cells although little work has been undertaken to identify and characterise its role (Cowan et al., 2020). In zebrafish models, expression of harmonin (USH1C) was shown to be necessary in Müller glial cells to support photoreceptor synaptic development and function (Phillips *et al.*, 2011). Indeed, USH1C expression within the larval retina was restricted to Müller glial cells. This observation is consistent with greater expression of Cdh23 at earlier timepoints in retinal development in wildtype murine retinae (Zhang *et al.*, 2020). Although beyond the scope of this review, it is notable that CLRN1 expression (associated with Usher type 3), was restricted to Müller glial cells in murine (Geller *et al.*, 2009) and human retina (Xu *et al.*, 2020a). It is unclear as to whether the USH1 proteins expressed in Müller glial cells relate to their potential as rod progenitor cells, or whether they serve a specific, and as yet undetermined function. Moreover, Müller cell dysfunction in USH1 may provide an additional mechanism through which photoreceptor degeneration occurs – although this remains unproven for *CDH23* and *PCDH15*. Expression of the monogenic retinal cadherinopathy genes within other retinal layers, such as CDHR1 within retinal ganglion cells, or USH1 proteins within the outer

plexiform layer/photoreceptor synapses (Reiners et al., 2006, Reiners et al., 2005) poses additional questions as to their roles in the inner retina (Berg et al., 2019). Original data comparing the expression of cadherins in different retinal cell types in *ex vivo* retinal tissue and retinal organoids is available (Cowan et al., 2020). Further investigation is required to determine the clinical significance of expression of retinal cadherins within the inner retina, including Müller glia cells.

The availability of an uncharacterised knockout Cdh3 mouse model presents an opportunity to identify and further characterise the associated retinal phenotype. If RPE degeneration is identified, and retinal function is found to be affected, there is an opportunity to develop and evaluate experimental therapies using the validated mouse model. The retinal phenotype in EEMS requires further characterisation using multimodal retinal imaging techniques and electroretinography. This may allow a direct comparison of retinal phenotypes between EEMS and HJMD. However, EEMS is a rare manifestation of *CDH3* variants and collection of relevant cases may be challenging. There appears to be significant phenotypic variation within reported EEMS patients with regards to the nature of hand and foot malformations (Kjaer et al., 2005). This may relate to various modifiers that may affect the expression of disease, independent of the *CDH3* variant. Moreover, it is possible that a different gene, or one linked to *CDH3* may be responsible for the limb abnormalities in EEMS.

Systemic expression of the four key retinal cadherins (Table 1) underlying the monogenic retinal cadherinopathies may explain their contribution to other non-ocular diseases. CDH23 has been implicated in the development of familial and sporadic pituitary adenoma (Zhang et al., 2017), as a poor prognostic marker in forms of cancer (Cao *et al.*, 2021), and in the susceptibility to Schizophrenia (Balan *et al.*, 2021). Similarly, PCDH15 variants have been implicated as a poor prognostic marker in ocular adnexal sebaceous carcinoma (Xu *et al.*, 2020b), copy number variants and rare SNP with psychiatric disorders (Ishii *et al.*, 2019, Ishizuka *et al.*, 2016), and SNPs with hyperlipidaemia

(Huertas-Vazquez et al., 2010). Although patients with USH1D and 1F do not undergo screening for such associations, these observations strongly suggest that these genes function beyond their known roles in the retina and inner ear. *CDHR1* and *CDH3* have also been reported to influence the behaviour and prognosis of various forms of cancer (Wang *et al.*, 2021, Taniuchi *et al.*, 2005, Liu *et al.*, 2020, Kumara *et al.*, 2017, Cao et al., 2021). Further elucidation of the mechanisms underlying these observations may be of relevance to their roles in retinal function and dysfunction.

CDHR1 is also expressed in brain tissue (Fu et al., 2018), within the pineal gland and the olfactory bulb. The *Pcdh21* promoter is used to drive transcription in mitral and tufted cells within the olfactory bulb; a Pcdh21-Cre recombinase mouse is used as a tool in studies of olfactory function (Nagai *et al.*, 2005). Although *CDHR1* is expressed in the olfactory bulb, there are no reports of hyposmia or anosmia in patients with biallelic *CDHR1* variants (Kato *et al.*, 2012, Nakajima *et al.*, 2001). However, this has not been studied directly. Formal olfactory testing suggested that USH1 patients have significantly better olfactory function compared to controls, although exhibit more rapid decline in olfactory function (Ribeiro *et al.*, 2016).

9. Therapeutic strategies for retinal degeneration from monogenic cadherinopathies

Identification and molecular characterisation of the retinal cadherinopathies has not only permitted the distinction of individual retinopathies (i.e. USH1 subtypes), but has also enabled therapeutic strategies targeted at individual genes or specific variants within a gene. Furthermore, in forms of retinal degeneration with common and well characterised pathophysiological pathways (i.e. USH1 genes), the targeting of a common disease pathway or molecule may represent a further plausible therapeutic approach.

In this section, we first identify ongoing clinical trials for retinal cadherinopathies, and retinitis pigmentosa for which patients with biallelic variants in *CDH23*, *PCDH15* and *CDHR1* may be eligible. Thereafter, we consider the most promising approaches to targeted genetic therapies for each retinal cadherinopathy with reference to the length of its DNA coding sequence (i.e. cDNA), expression within the retina or RPE, the rate of retinal degeneration of the associated clinical phenotypes, and the distribution of variants across the gene. Gene replacement, gene editing (DNA or RNA), anti-sense oligonucleotides (ASO) therapies, and non-genetic ocular and systemic therapies are considered. Thereafter, regenerative strategies are briefly summarised: stem cells (retinal and RPE), optogenetics and electronic retinal implants which may be of relevance for end-stage outer retinal degeneration from retinal cadherinopathies.

9.1 – Clinical trials

There are currently three registered (ClinicalTrials.gov) interventional trials for Usher syndrome type 1, although only one trial – a placebo-controlled study of an undisclosed oral drug - is suitable for any genetic form of USH1 (NCT04355689). Patients with Usher syndrome may also be eligible for some interventional clinical trials for retinitis pigmentosa that are not gene specific (i.e. NCT: NCT04636853) . However, there are no registered clinical trials that aim to modify genes involved in monogenic retinal cadherinopathies. A single prospective natural history study has started for patients with Usher syndrome type 1F (*PCDH15*) (NCT: NCT04765345).

9.2 Retinal gene therapy

Gene therapy aims at supplementing absent or deficient genetic sequences within cells. For monogenic disorders, gene supplementation aims to treat the underlying cause of the disease. Moreover, in terminally differentiated cells, such as photoreceptors and RPE cells, stable long-term expression of a gene may be achieved with a single intervention.

Vectors are used to deliver genetic sequences to target cells. Adeno-associated virus (AAV) is the most commonly used vector in human gene therapy clinical trials as it is non-pathogenic, non-integrating, and non-dividing in the absence of viral co-infection. Moreover, AAV is capable of transducing numerous cell types (including photoreceptors and RPE), and may produce stable expression of a gene through the formation of episomes within the cell. The main limitation of AAV is the limited sequence length that can be packaged within its genome (4.7kb), including promoter and other regulatory elements that modify gene expression and stabilise the expressed transcripts. *CDHR1* (2.58kb) and *CDH3* (2.49kb) are genes underlying monogenic retinal cadherinopathies whose cDNA sequences fall within the packaging limit of single AAV particles. However, the cDNA sequences of *CDH23* (10kb) and *PCDH15* (5.89kb) exceed the capacity of AAV. The use of double and triple vector AAV systems have been described which would be sufficient to package the *CDH23* and *PCDH15* coding sequences (Maddalena *et al.*, 2018) (Carvalho *et al.*, 2017). However, the efficiency of dual and triple AAV strategies are reduced when compared to single AAV transduction systems (Carvalho et al., 2017, Trapani *et al.*, 2015) since it is dependent on the successful concatermerisation of two to three overlapping peptide sequences within the target cell. Furthermore, for *CDH23* and *PCDH15*, the absence of a retinal phenotype in the relevant rodent models and difficulty in expressing photoreceptor-specific transgenes in relevant cell lines presents a challenge to pre-clinical vector development of gene replacement strategies.

Numerous non-viral delivery systems have been described, although are limited by the efficiency of nuclear localisation, and the stability of long-term transgene expression (Charbel Issa and MacLaren, 2012). Moreover, within the retina, numerous non-viral vectors are toxic, in part because solutions used to permeabilise cell membranes are toxic to photoreceptor OS discs. Other gene transfer

methods may be difficult to access and apply to retinal tissue, or efficacious only in newborn animals, such as electroporation.

9.2.1 Gene replacement for *CDHR1*-associated cone-rod dystrophy

Since *CDHR1*-associated retinal dystrophy occurs from biallelic loss of function variants, the simplest therapeutic strategy for *CDHR1*-associated retinal dystrophy is gene supplementation of the full length human *CDHR1* coding sequence targeting both cone and rod photoreceptors. The human *CDHR1* cDNA sequence does not have a repetitive structure or significant number of low-use codons; codon-optimisation is not necessary. An efficient photoreceptor-specific promoter is necessary to express the transgene in both cone and rod photoreceptors, with or without additional regulatory elements (i.e. polyadenylation tail) and/or enhancing sequences to achieve optimum expression of CDHR1. Detailed *in vivo* analysis of the *Cdhr1*$^{-/-}$ mouse with deep phenotyping over 15-months showed progressive rod and cone photoreceptor degeneration, recapitulating the characteristics of the human retinal disease and validating this animal model for the pre-clinical development of *CDHR1* gene therapy (Yusuf et al., 2021).

The absence of a cell line that expresses transgenes driven by a photoreceptor specific promoter would necessitate retinal organoids or an *in vivo* assessment in the *Cdhr1*$^{-/-}$ mouse in order to test the vector. Vectors may be delivered by intravitreal or sub-retinal injection; the latter has been associated with less intraocular inflammation since a lower overall dose is required to target photoreceptors (Ochakovski *et al.*, 2017). *In vivo* OCT imaging and electroretinography may represent objective structural and functional outcome measures to determine the optimum dose, and thereafter, the efficacy of gene supplementation. Potential challenges in *CDHR1* retinal gene therapy involve AAV related immunogenicity and possible immune responses to the *CDHR1* transgene following successful photoreceptor transduction since the protein is membrane bound. Dose-

dependent immune responses have been reported in phase 1 clinical trials involving the delivery of sub-retinal AAV-mediated gene therapy, reviewed extensively elsewhere (Bucher *et al.*, 2020).

Further research is required to characterise the natural history of human *CDHR1*-associated cone-rod dystrophy in order to determine the optimum timing for intervention and to identify clinically relevant and sensitive outcome measures to help justify and design future clinical trials. This may necessitate prospective clinical trials in patients with biallelic *CDHR1* variants. It should be noted that CDHR1 is one of the more common causes of cone, cone-rod and macular dystrophy when hypomorpic variants are included (Birtel *et al.*, 2018).

9.2.2 Gene replacement for *CDH3*-associated retinopathy

Identification of progression of macular atrophy in HJMD patients suggests a therapeutic window for intervention.(Hull et al., 2016) Furthermore, congenital hypotrichosis is a clear phenotypic feature that may lead to the early diagnosis of HJMD and thereby facilitate early intervention. The coding sequence (cDNA) of *CDH3* is 2,490bp which falls within the packaging limit of adeno-associated virus.(Singh *et al.*, 2016) Moreover, AAV2 has been shown in pre-clinical studies and clinical trials to effectively transduce RPE cells (MacLaren *et al.*, 2014), where *CDH3* is expressed. Recombinant AAV2.hCDH3 gene therapy using an RPE-specific promoter is therefore the most plausible therapeutic approach, which would be independent of the specific variant in all individuals with truncating variants with both EEMS and HJMD.

A *Cdh3* knockout mouse (P-cadherin knockout) has been created through insertion of the PGK-neomycin targeting cassette within the Cdh3 open reading frame. Ocular examination of the *Cdh3*$^{-/-}$ homozygous mouse has not been undertaken. Although the murine retina does not contain an anatomical macula, RPE dysfunction may be detected using structural (such as *in vivo* fundus

autofluorescence, and optical coherence tomography), and functional methods (using electrophysiological testing). Furthermore, histological analysis may identify evidence of RPE degeneration in *Cdh3*$^{-/-}$ mice. If a retinal phenotype is evident, the *Cdh3*$^{-/-}$ mouse could form a useful model for the pre-clinical development of therapeutics. However, other approaches, for example, using retinal organoids or RPE cells produced from human induced pluripotent stem cells from a *CDH3*$^{-/-}$ patient may aid in the development of molecular therapies. A further challenge, given the rarity of macular dystrophies due to *CDH3* variants, is recruitment to adequately power a clinical trial of a novel therapeutic.

9.3 Gene editing

Precise gene-editing is possible using the CRISPR-Cas 9 system, is a powerful technique in which an endonuclease, present in bacteria as part of the adaptive immune system to viral infection (Jinek *et al.*, 2012), can be programmed to modify specific DNA sequences in living eukaryotic cells. CRISPR-Cas 9, which cuts double-stranded DNA in its natural form, has been modified to broaden the scope of editing activity and relax protospacer-adjacent motif (PAM) specificities to increase the number of therapeutic targets. For example, base-editing describes the use of a deaminase linked to a nickase in which a single catalytic site in the Cas9 enzyme is deactivated, permitting the correction of single nucleotide transition variants without creating breaks in double-stranded DNA (Rees and Liu, 2018). Prime editing is a further powerful modification of the CRISPR system in which the tracrRNA functions to specify the target site and also as a template for DNA repair following reverse transcription (prime editing guide or pegRNA) (Anzalone *et al.*, 2019). This technique is capable of correcting a longer DNA sequence, and a single optimised pegRNA could potentially correct a number of clustered pathogenic variants in a DNA sequence. The *in vivo* applications of genome editing are extensively reviewed in (Yanik *et al.*, 2017) and additional RNA-targeting gene therapy applications within the retina in (Fry *et al.*, 2020). RNA-targeting may be theoretically safer since

the endonuclease (Cas13) is active against expressed transcripts rather than DNA, although sustained expression of editing proteins and RNA sequences is necessary.

Gene editing is a promising therapeutic strategy for retinal cadherinopathies, particularly for USH1 subtypes (*CDH23* and *PCDH15)* whose cDNA sequences exceed single recombinant AAV encoding. Most disease-associated variants in *CDH23* and *PCDH15* are single base transition or transversion mutations, or small insertions or deletions (Supplementary Tables 3&4) which are amenable to CRISPR-Cas9 base- or prime-editing. However, each disease-associated variant will require assessment to determine whether a suitable PAM site is present, relevant to validated Cas9 orthologues that function efficiently in prime- or base-editing systems. However, near PAM-less Cas9 variants have been engineered which do not have the same constraints of sequence recognition adjacent to a DNA target (Walton *et al.*, 2020, Zhang and Zhang, 2020). Pre-clinical validation of candidate sgRNA/pegRNAs is required, which require packaging all-in-one in AAV particles for optimal *in vivo* delivery. A significant amount of work is required to clone the relevant sequences, optimise candidate sgRNAs/pegRNAs and determine both on-target and off-target editing efficiencies *in vitro*. Consequently, only common pathological variants in these genes are viable therapeutic targets. The first phase 1/2 clinical trial of AAV-delivered CRISPR molecules to the retina of patients with LCA due to *CEP290* variants is currently ongoing (Clinicaltrials.gov no. NCT 03872479), although further details of the vector are not available. Further clinical trials involving patients with disease associated variants in large genes are anticipated using CRISPR-Cas9 DNA or RNA editing systems, such as *CDH23* and *PCDH15*.

Prime-editing is a gene-editing technique that is capable of replacing a DNA sequence. In *CDH23*, a clustering of disease-associated variants associated with USH1D exists in the 20th extracellular cadherin repeat with six truncating variants within a 67-codon interval (Figure 5). Consequently, a

single therapy would be relevant to all individuals with USH1D who have at least one of the six truncating variants on at least one allele (Anzalone et al., 2019).

CRISPR base-editing strategies may also represent an attractive therapeutic approach for individuals with *CDHR1*-associated late-onset macular dystrophy resulting from presumed hypomorphic variants. Efficient targeting of a single mutant allele would be theoretically sufficient since there is no apparent phenotype in heterozygous carriers of *CDHR1* variants, or in the heterozygous mutant mouse (Rattner et al., 2001). Hypomorphic variants are predominantly missense variants, although a frequent synonymous variant (c.783G>A$_{CDHR1}$) is particularly common in European population (mean allele frequency of 0.31%). Most of these variants are amenable to adenine base editing. In some individuals, who have a severe functional variant on the second allele, variable peripheral retinal degeneration may co-exist (Charbel Issa et al., 2019). In this group, assuming no interaction between the *CDHR1* transgene and the mutant hypomorphic transcript, *CDHR1* gene supplementation therapy may be of benefit. However, a dominant negative effect of the expressed transgene on mutant transcripts is a possibility. Further investigation is required to determine the effect of *CDHR1* gene therapy on the expression of constitutively expressed mutant transcripts, either hypomorphic-null or biallelic hypomorphic variants. Primary *ex vivo* cell lines from patients (i.e. fibroblasts) may be used to further investigate gene supplementation in this patient group, with the *CDHR1* transgene driven by a non-specific promoter, or the therapeutic application of CRISPR gene editing strategies where sequencing of transcripts may form the main outcome measure.

9.4 Antisense oligonucleotides

Antisense oligonucleotides (ASOs) are single-stranded nucleic acids sequences that may be exploited in monogenic disease through several functional mechanisms: prevention of translation through binding to pre-mRNA, modification of a transcript through the inclusion or exclusion of specific

exons, degradation of pre-mRNA by RNase H, or interference with the upstream untranslated region or downstream polyadenylation tail (Silva *et al.*, 2020). Gene knockdown is relevant for dominant disease caused by a dominant negative disease mechanism, but not haploinsufficiency. In the retina, ASOs have been shown to prevent the coding of pseudo-exons leading to transcript truncation (reviewed extensively (Xue and MacLaren, 2020)). This has been shown to correct splicing defects in models of CEP290 associated Leber congenital amaurosis (Dulla *et al.*, 2018) leading to a follow-on phase 1 clinical trial in human subjects (ClinicalTrials.gov no. NCT03140969) within promising interim findings (Cideciyan *et al.*, 2019).

Although deep intronic variants that produce a pseudo-exon have not been described for any retinal cadherinopathy, ASOs directed against canonical splice sites may be used to force pre-mRNA splicing to in-frame exon skipping in order to abrogate the effect of a functionally severe variant within the skipped exon. The therapeutic potential of ASO could be expanded by the skipping of multiple exons where the reading frame of individual exons is out of frame. This principle has been used to treat pathological variants in Duchenne's Muscular Dystrophy in which parts of the encoded protein is highly repetitive (Echigoya *et al.*, 2018). Although cadherin repeats are not identical, particular extracellular cadherin repeats in CDH23 (27 repeats) and PCDH15 (11 repeats) may be functionally less important than others, and circumvented whilst retaining protein function. However, this approach may be more relevant to truncating variants rather than pathological missense variants which often occur in functional domains of the protein. ASO-mediated exon skipping may target more than one pathological variant within a given set of exons, and a single therapy could potentially circumvent more than one pathological allele. These ASO-mediated approaches may convert a severe phenotype of retinal degeneration into a milder one, or may reduce the rate of photoreceptor degeneration.

In order to translate ASOs to treat retinal cadherinopathies, the functional effects of clinically validated variants affecting canonical splice sites may help to predict which exon-intron boundaries to target across the four genes. Furthermore, modelling the functional consequences of exon skipping in *CDH23* is a necessary prerequisite to determine which set of variants may be circumvented. Although conventional experimental *and in silico* methods can predict protein folding, more recently, artificial intelligence (AlphaFold) has been shown to outperform *in silico* predictions of protein folding where no similar structure is known, and with similar efficacy to experimental approaches (Jumper *et al.*, 2021). *In vitro* splicing assays may be used to demonstrate the effect of ASO on the resulting transcripts. These tools may be used to apply ASO in patients with retinal cadherinopathies. The functional effects of ASO are not dependent on the size of the gene, and could be of therapeutic benefit in *CDH23* and *PCDH15*-associated USH1 which are too large for single-vector AAV-encoding. Accordingly, the report of *CDH23* patients with variants affecting canonical splice sites or are predict to result in in-frame exon skipping are important in order to further characterise the functional effects of potentially targetable variants (Supplementary Table 3) (Menghini *et al.*, 2019).

9.5 Non-genetic ocular or systemic therapies

Systemically administered therapies may be of benefit to patients with retinal cadherinopathies: for example, an orally administered experimental anti-oxidant drug, a GMP-grade of N-acetylcysteine amide, is currently under phase 1/2 clinical trial in patients with all forms of Usher syndrome (Clinicaltrials.gov identifier NCT04355689) (Kong *et al.*, 2021). There is some limited pre-clinical evidence that antioxidant administration may reduce cone death in USH1 murine models (Trouillet *et al.*, 2018).

Aminoglycosides, and other closely related derivate molecules termed translational readthrough inducing drugs (TRIDs), have been shown to reduce the fidelity of translation and allow read-through of truncating transcripts (as reviewed by (Nagel-Wolfrum *et al.*, 2016)). This approach has been shown to suppress nonsense variants in *PCDH15* and Harmonin *in vitro* and *ex vivo* (Rebibo-Sabbah *et al.*, 2007, Goldmann *et al.*, 2010). Although the degree of suppression of mutant transcripts varied depending on the sequence context around the stop codon, maximum suppression levels of up to 91% were demonstrated. However, aminoglycosides may be limited by their toxicity when administered inside the eye, have limited bioavailability within the retina from systemic administration, and potential off-target effects on general translation within photoreceptor cells and other tissues. Currently, there are no clinical trials of these agents for inherited retinal degenerations, although phase 2 studies in cystic fibrosis and inherited renal diseases have been registered (Clinicaltrials.gov).

Patients with retinal degeneration from the retinal cadherinopathies may develop cystoid macular oedema. Intravitreal steroid therapies (Scorolli *et al.*, 2007, Park *et al.*, 2020, Mansour *et al.*, 2018), and topical and systemic carbonic anhydrase inhibitors are often used in the treatment of degeneration cystoid macular oedema. Posterior subcapsular cataract formation is common in retinitis pigmentosa which can be removed surgically when indicated.

9.6 Therapeutic strategies for end-stage outer retinal degeneration

In patients with end-stage retinal degeneration from retinal cadherinopathies, there are three main therapeutic approaches: optogenetics in which ectopic expression of opsins may restore light responses following loss of photoreceptors (reviewed in (McClements *et al.*, 2020, Botto *et al.*, 2021), retinal stem cell therapies (reviewed in (Wiley *et al.*, 2015)), or retinal prostheses in which an artificial light-sensing system relays electrical signals to the inner retina. While the first 2 approaches

are currently only investigated in clinical trials, some electronic implants are approved for clinical use in Europe and the US.

Optogenetic therapies express light-sensing proteins ectopically within the retina in advanced outer retinal degeneration – typically opsins within ON-bipolar cells (Yue *et al.*, 2016, Botto et al., 2021). Using the target cells apparatus, electrical responses are generated and propagated by the intact inner retina to the primary visual cortex. Visual improvement has been reported following treatment in blind patients following therapy (Sahel *et al.*, 2021). For advanced inherited retinal degeneration, four phase 1/2 clinical trials are registered (Clinicaltrials.gov) for different optogenetic retinal gene therapy products, although none have reached phase 3 clinical trial or are approved for clinical use.

Three retinal cadherinopathies (*CDHR1*, *CDH23* and *PCDH15*) are characterised by primary photoreceptor degeneration. Despite promising pre-clinical studies demonstrating improved visual function following delivery of photoreceptor precursors (MacLaren *et al.*, 2006, Singh *et al.*, 2013) there is not yet an approved stem cell therapy for retinitis pigmentosa. A number of phase 1/2 clinical trials are recruiting patients with advanced retinitis pigmentosa; the field is reviewed extensively in (Singh *et al.*, 2020). *CDH3*-associated retinopathy is characterised by a primary RPE degeneration at the macula with secondary loss of photoreceptors. Replacement of RPE through the injection of stem cell suspensions, or implantation of tissues sheets cultured *in vitro* using scaffolds has been a research objective in the treatment of dry age-related macular degeneration (Binder *et al.*, 2007). Clinical studies reported excellent visual outcomes following implantation of embryonic stem cell-derived RPE monolayers (da Cruz *et al.*, 2018). For patients with *CDH3*-associated retinopathy, localised RPE transplantation within the macula may similarly preserve central visual function. The stem cells may be derived from either embryonic or pluripotent sources. Cells derived from a patient

with biallelic *CDH3* variants would require *ex vivo* correction of at least one *CDH3* variant prior to implantation.

Electronic retinal implants involve long surgical procedures with relatively high risk of complications; however, some patients may experience excellent visual outcomes (Cehajic Kapetanovic *et al.*, 2020). The AMS subretinal prosthesis was shown to improve vision in 5 of 6 participants with retinitis pigmentosa to 24 months (Edwards *et al.*, 2018). The Argus II epiretinal prosthesis is approved in both Europe and the US (Ho *et al.*, 2015), with safety data beyond 5 years (da Cruz *et al.*, 2016).

10. Conclusions and future directions

In 1997, a previous review in *the journal* summarised the physiological function, regulation and pathophysiological relevance of previously identified retinal cadherins (Grunwald, 1996). At that time, only around 30 of the 271 genes now identified as causes of retinal disease (https://sph.uth.edu/retnet/home.htm) had been discovered. Moreover, since cadherins expressed in photoreceptors had not been identified, variants in genes encoding cadherins were not predicted to cause primary photoreceptor degeneration. Instead, it was considered that dysfunction of retinal cadherins could contribute to cell detachment and migration relevant to the development and progression of retinal tumours and proliferative vitreoretinopathy (Grunwald, 1996).

The identification of *CDH3* (2001), *CDH23* (2001), *PCDH15* (2001) and *CDHR1* (2010) as monogenic causes of retinal degeneration – the retinal cadherinopathies – has transformed the understanding of the function of cadherins within the retina. *CDH3*, the dominant cadherin expressed in the RPE, aligns to the structure and function of a classical cadherin through homophilic interactions producing calcium-dependent cell-to-cell adhesion between adjacent RPE cells as part of

the adherens junction. However, *CDHR1*, *CDH23* and *PCDH15* are atypical cadherins which serve specialised functions in vertebrate photoreceptors. Rather than mediating cell-to-cell adhesion like classical cadherins, these atypical cadherins achieve and maintain complex structural arrangements between different regions of the same cell. More specifically, *CDHR1*, *CDH23* and *PCDH15* regulate the morphogenesis of the photoreceptor OS discs; absence of any of these three cadherins results in dysmorphic photoreceptor OS, with subsequent photoreceptor cell death. *CDHR1*, *CDH23* and *PCDH15* are also expressed in other cell types within the retina although their roles within retinal ganglion cells and/or Müller glia are as yet unknown. Moreover, numerous other cadherins are expressed within the retina, contributing to retinal development, tissue organisation and other processes; their mutational effects are currently unknown.

Murine models have proven valuable in characterising the function of *CDHR1* within the retina, (Yusuf et al., 2021) further refined by ultrastructural and biochemical analysis in wildtype mice (Burgoyne et al., 2015, Rattner et al., 2001). *CDHR1* forms physical connections between nascent, evaginating OS discs and the periciliary ridge of the IS of rod and cone photoreceptors. The characterisation of genes that regulate expression of retinal cadherins are candidate genes for unsolved cases of retinal degeneration; *ADAM10* and *SFRP1* may regulate cleavage of the *CDHR1* ectodomain (Cisneros et al., 2020) necessary to uncouple mature OS discs from the IS throughout life, although confirmation is required. The identification of the IS binding partner of CDHR1 may provide an additional candidate gene for retinal degeneration and provide important information to further unravel the molecular basis of OS disc morphogenesis (Goldberg *et al.*, 2016). Genotype-phenotype associations reveal a distinction between generalised retinal dystrophy with biallelic null variants in *CDHR1* and late-onset macular dystrophy due to presumed hypomorphic variants on at least one allele. Why are macular photoreceptors predisposed to degeneration with mild

perturbations in the *CDHR1* ectodomain? (Yusuf *et al.*, 2020) The identification of the IS binding partner may help to answer this question.

Further studies are required to determine the genetic prevalence of *CDHR1*-associated late-onset macular dystrophy which may masquerade as age-related atrophic macular degeneration and is likely to be underdiagnosed. The coding sequences of both *CDH3* and *CDHR1* coding sequences are sufficiently small to be packaged into recombinant AAV vectors and mouse models are available to investigate the efficacy of a candidate therapeutic on retinal structure and function. The *Cdh3*$^{-/-}$ mouse requires validation through detailed retinal phenotyping. *CDHR1*-associated cone-rod dystrophy and late-onset macular dystrophy may be amenable to retinal gene therapy.

Elucidating the tissue localisation and function of cadherins underlying USH1 subtypes in the retina is complicated by the absence of calyceal processes in rodents around which all USH1 proteins are expressed. Mouse models of USH1, including *Cdh23* and *Pcdh15*, generally do not recapitulate the clinical phenotype of RP seen as part of USH1, causing retinal dysfunction but not degeneration. However, immunohistochemical analysis of non-human primate and human retinal tissue localises USH1 proteins to the calyceal processes; microvilli-like projections that envelop the proximal photoreceptor OS and appear to prevent lateral outgrowth of the OS in most vertebrates. The absence of calyceal processes in rodents is thought to underpin the absence of an RP phenotype in USH1 knockout murine models. Knockdown of *Pcdh15* results in marked lateral outgrowth in the region of the proximal OS discs enclosed by the calyceal processes. Taken together, these observations suggest that the retinal cadherins *CDH23* and *PCDH15* function in the development and maintenance of photoreceptor OS disc alignment, that appears more critical in rods.

However, the precise arrangement of the USH1 network has not been characterised in detail in the retina. In the hair cells of the inner ear, PCDH15 and CDH23 interact directly to form tip links between adjacent developing stereocilia and between stereocilia and the kinocilium, which gates mechanotransduction. Since the calyceal processes are considered as morphologically analogous structures, one hypothesis is that either PCDH15 homodimers, CDH23 homodimers or PCDH15-CDH23 heterodimers may form physical connections between the OS membrane and the calyceal processes which link to their actin cytoskeleton, through other USH1 proteins (Schietroma et al., 2017). The tip links within the inner ear are linked to the actin cytoskeleton of stereocilia by other USH1 proteins (Sans, Harmonin, MYO7A). Further, detailed characterisation of the retinal USH1 protein network is required; the USH1 protein interactions which have been characterised in the inner ear permits a hypothesis-based approach to mapping of the retinal USH1 network. Elucidation of the USH1 network may confirm a common disease pathway from which potential gene-independent therapies may be designed for USH1-related RP. Moreover, functional domain mapping of USH1 proteins may permit a more accurate prediction of phenotype based on variants within the relevant genes. Proteins identified as part of the USH1 network may unravel the unidentified genes within the identified USH1 loci. Further work is required to determine the roles of cadherins in Müller glia and retinal ganglion cells.

The coding sequence of *CDH23* and *PCDH15* are too long to package in recombinant AAV vectors. Gene editing technologies that act at the DNA or RNA level exist, and the targeting of variants in large genes such as CDH23 and PCDH15 is now plausible. The absence of retinal phenotypes in rodent models may reduce the value of structural and functional outcome measures following intervention. However, on-target efficiency and off-target assessment of gene-editing in strains that model common variants in these genes would be useful to demonstrate proof-of-principle in murine models of USH1. The rapid evolution of base-editing technologies permits safer DNA and RNA

editing with more efficient deaminases that produce fewer off-target effects; packaging within the AAV genome appears to be the limiting factor in the application of these technologies.

In summary, the discovery of the retinal cadherinopathies has expanded our knowledge of the contributions of cadherins to retinal structure and function. Although further molecular characterisation is required, the retinal cadherinopathies are potentially treatable forms of retinal degeneration: *CDHR1* and *CDH3* with gene replacement strategies and *CDH23* and *PCDH15* with gene editing strategies. Genes regulating retinal cadherin expression, and other cadherins expressed in the retina may represent candidates for unsolved cases of retinal degeneration.

Figures

Figure 1: The cadherin superfamily. Membership in the cadherin superfamily requires the presence of at least two extracellular cadherin repeats (EC, blue). The ~100 superfamily members are categorized based on the primary amino acid sequence of EC1 into the cadherin main branch (top) or cadherin related branch (bottom). Exemplars of the subgroups within these major divisions illustrate the diversity of the superfamily both in the number of EC repeats (ranging from 2 to 34) and the presence of other common extracellular domains including EGF-like (pink) and Laminin-G (green) domains. Cytoplasmic domains are divergent between the subgroups, as indicated.

Figure 2: Cadherin superfamily cytoplasmic interactions. Five subgroups of the cadherin superfamily with essential functions in the retina have diverse cytoplasmic interactions. The classical cadherins, including Type I cadherins like CDH3 and Type II cadherins like CDH6, interact directly with p120-catenin via a juxtamembrane binding site and β-catenin near the C-terminus. β-catenin recruits α-catenin, providing a link to the actin cytoskeleton through direct binding or additional actin-binding molecules (pink circles). PCDH15 directly binds the long isoform of whirlin/MYO15A, or harmonin/MYO7A complexes to connect to the actin cytoskeleton. CDH23 forms a stable complex with harmonin, sans, and MYO7A to link adhesion to the actin cytoskeleton. CDHR1 directly interacts with the pentaspan transmembrane protein Prom-1, which regulates CDHR1 localization to photoreceptor discs. Additional interactions are not yet described. The mechanisms through which the cPcdhs promote neuronal survival and self-avoidance are unknown. However, γC4 is the only isoform essential for survival, suggesting isoform-specific interactions, and signaling through Fak or Pyk2 are candidates for self-avoidance.

Figure 3: Localization of essential cadherin-superfamily-mediated adhesion in the inner and outer retina. A) Cadherins are important for establishing the laminar organization of the retina. In the RPE, CDH3 (green) forms adherens junctions crucial for maintaining the integrity of the epithelium. **B**) In photoreceptors, CDHR1 (red) forms physical connections between the leading edge of nascent outer segment discs and the periciliary ridge of the inner segment, supporting horizontal disc elongation. More distally, cleavage of the CDHR1 ectodomain separates it from the inner segment. (C) Heterophilic adhesion between PCDH15 (orange) and CDH23 (green) link calyceal processes to each other and the plasma membrane of the OS. ILM = inner limiting membrane; NFL = nerve fibre layer; GCL = ganglion cell layer; IPL = inner plexiform layer; INL = inner nuclear layer; OPL = outer plexiform layer; ONL = outer nuclear layer; ELM = external limiting membrane; RPE = retinal pigment epithelium.

Figure 4. Distribution of pathological sequence variants and retinal imaging characteristics in *CDHR1*-associated retinal degeneration. (A) Variants relate to reference sequence: NM_033100.4. Bold font indicates likely hypomorphic variants associated with late-onset macular dystrophy. Red – frameshift, stop gain, start lost; blue - splice site; green – missense; black – in-frame deletions, synonymous variants. Supplementary Table 2 presents nucleotide change, domain, allele frequency, phenotype and source for each *CDHR1* variant. **Middle panel: CDHR1-associated cone-rod dystrophy (B)** Widefield fundus colour imaging appears generally unremarkable appearance in early disease. **(C)** Widefield fundus autofluorescence imaging demonstrates an irregular hypoautofluorescent foveal signal (shown in detail in **(D)** with generalised hyperautofluorescence throughout the macula. There is a granular appearance to the mid-peripheral retina. **(E)** Macula OCT imaging shows generalised thinning of the outer nuclear layer with peripheral loss of the ellipsoid zone. Outer retinal debris can be seen overlying the RPE at the fovea.

Lower panel: CDHR1-associated late-onset macular dystrophy: **(F)** Colour fundus photograph showing a central areolar choroidal dystrophy phenotype with RPE and retinal atrophy within the macula with a well demarcated border. **(G)** Fundus autofluorescence imaging showing total loss of the RPE with a speckled hyperautofluorescent border, beyond which the surrounding retina appears unaffected. **(H)** OCT imaging of the macula shows loss of the RPE and outer retina centrally with choroidal hypertransmission and normal retinal architecture peripherally.

Figure 5. Distribution of pathological sequence variants and retinal imaging characteristics in *CDH23* associated with USH1D. (A) Variants relate to reference sequence: NM_022124.6. Red – frameshift, stop gain; blue - splice site; green – missense; black – in-frame deletions, synonymous variants. Supplementary Table 3 presents nucleotide change, domain, allele frequency, phenotype and source for each *CDH23* variant. **(B)** Widefield fundus pseudocolour image in this 18-year-old male individual demonstrates peripheral retinal thinning with mid-peripheral pigment migration and mild arteriolar attenuation. **(C)** Widefield fundus autofluorescence imaging showing patchy mid-peripheral hypoautofluorescence with a hyperautofluorescent ring at the leading edge of centripetal outer retinal degeneration. **(D)** Macular OCT shows preservation of the fovea architecture and outer retinal morphology, except at the limits of the image (arrowhead) where loss of the photoreceptor OS is seen, coincident with the hyperautofluorescent ring seen in B.

Figure 6. Distribution of pathological sequence variants and retinal imaging characteristics in in *PCDH15* associated with USH1F. (A) Variants relate to transcript ID, NM_033056.3. Red – frameshift, stop gain; blue - splice site; green – missense; black – in-frame deletion. Supplementary Table 4 presents nucleotide change, domain, allele frequency, phenotype and source for each *PCDH15* variant. **(B)** Widefield pseudocolour fundus imaging in this 53-year-old female individual demonstrates widespread mid-peripheral pigment migration extending within the macular arcades

associated with optic disc pallor and generalised arteriolar attenuation. **(C)** Widefield fundus autofluorescence imaging shows widespread hypoautofluorescence due to confluent loss of the retinal pigment epithelium leaving a small island of hyperautofluorescence nasal to the fovea **(D)** Macular OCT imaging shows extensive outer retinal degeneration involving the fovea. An island of remaining RPE is seen nasal to the fovea.

Figure 7. Distribution of pathological sequence variants and retinal imaging characteristics in *CDH3*-associated macular dystrophy. Upper panel: Variants relate to transcript ID, NM_001793.6. Variants associated with a clinical phenotype of EEMS are indicated in bold typeface. Red – frameshift, stop gain; blue - canonical splice site; green – missense; black – in-frame deletions. Supplementary Table 5 presents nucleotide change, domain, allele frequency, phenotype and source for each *CDH3* variant. Lower panel is reproduced with permission from Sci Rep, 2016 (Singh et al., 2016). **(A)** Hypotrichosis **(B,C)** Fundus photographs of the right and left eyes showing atrophy (arrowheads) and pigment clumps pigmented clumps of retinal epithelial cells (black arrow). **(D&E)** Fundus autofluorescence imaging showing hypoautofluorescence corresponding to loss of the RPE with surrounding hyperautofluorescence **(F&G)** Macular OCT imaging showing the leading edge of degeneration (upwards arrowhead) with RPE clumps in the subretinal space indicated by downward arrows.

Supplementary Figure 1. Comparison of retinal expression of genes underlying the retinal cadherinopathies.

Figure is generated using data from Cowan et al, 2020 (Cowan et al., 2020) using *ex vivo* human retinal tissue, derived from Table S2. Expression values are normalised to 10,000 transcripts counts per cell type.

Tables

Table 1. The retinal cadherins.

Gene [NCBI gene ID]	Chromosomal location and length	cDNA length (bp)	Protein length (aa)	Cadherin repeats	Tissue expression (RNA)	Retinal tissue expression	Retinal phenotype	Systemic features	Interacting proteins
CDHR1 [92211] Synonyms: *CORD15, PCDH21, PRCAD, RP65, KIAA1775*	10q23.1 24,987bp	2,580 17 exons NM_033100.4	859 Q96JP9-1	6 (Non-classical)	Skin fibroblasts *Retina* Basal ganglia Gastrointestinal tract Natural Killer cells Pons/ medulla Cerebral cortex Olfactory region	Photoreceptors Ganglion cells	Cone-rod dystrophy Retinitis Pigmentosa Macular dystrophy	None reported	PROM1 Enzyme catalysing cleavage of ectodomain proposed as ADAM10 Inner segment binding partner unknown Siah1 (proteosomal degradation)
CDH23 [64072]	10q22.1 420kb	10,065 70 exons NM_022124.6	3,354 Q9H251-1	27 (Non-classical)	*Retina* Pons/ medulla Ovary Parathyroid gland Monocytes Cerebellum Pancreas Adipose tissue	Photoreceptors Cochlea	Retinitis Pigmentosa	Usher Syndrome Type 1D	Cadherin repeats 1 and 2 mediate calcium-dependent heterophilic interaction with PCDH15.
PCDH15 [65217] Synonyms: *CDHR15, DFNB23, USH1F (Cdh5 in c. elegans)*	10q21.1 980kb	5,889 33 exons NM_033056.3	1,955 Q96QU1-1	11 (Non-classical) (3-11 depending on isoform)	Adrenal gland *Retina* Kidney Cerebral cortex Hippocampal formation Amygdala Pons/ Medulla Thalamus	Photoreceptors	Retinitis Pigmentosa	Usher Syndrome Type 1F	Interaction with CDH23

CDH3 [1001]	16q22.1 82,660bp	2,490 18 exons NM_001793.6	829 P22223-1	5 (Classical)	Thymus *Retina* Ovary Tongue Esophagus Skin Tonsil Pons and medulla	RPE	Macular dystrophy	HJMD EEMS	Dimerises with CDH3

Table 2. Clinical features of the retinal cadherinopathies

Gene	Retinal phenotype	Onset	Symptoms at onset	Fundoscopy	OCT	FAF	Colour vision	Visual fields	Full field ERG
CDHR1	Cone-rod dystrophy	Variable (<30 years)	Cone: glare, colour vision deficit, abnormal central vision Rod: Poor night vision, visual field deficit	Abnormal foveal reflex Macular atrophy Peripheral retinal degeneration	Generalised thinning of ONL without foveal preservation Loss of EZ reflectivity	Enlargement of foveal hypoautofluorescence	Severely affected	Generalised constriction with central scotomas	Large reductions in cone and rod response amplitudes
	Macular dystrophy	~40s	Difficulty reading Blurred central vision Central scotomas	Flecks in parafovea Atrophic parafovea Geographic atrophy	Outer retinal degeneration in the parafovea Sparing fovea initially	Flecks in parafovea Atrophic parafovea Geographic atrophy	May be normal in early disease	Central scotomas	Normal or borderline full-field ERG responses; abnormal in late disease Abnormal pERG
	RP	Unk	Unk	Pigment migration Vascular attenuation Waxy disc pallor	Peripheral outer nuclear layer thinning Later macular involvement	Peripheral hypoautofluroescence, spreading centripetally	Preserved initially	Progressive visual field constriction	Full field: Rod dysfunction with later cone involvement
CDH23	RP	First decade	Night vision problems Visual field constriction	Pigment migration Vascular attenuation Waxy disc pallor	Peripheral outer nuclear layer thinning Later macular involvement	Peripheral hypoautofluroescence, spreading centripetally HyperAF ring may be present	Preserved initially	Progressive visual field constriction	Full field: Rod dysfunction with later cone involvement
PCDH15	RP	First decade	Night vision problems Visual field constriction	Pigment migration Vascular attenuation Waxy disc pallor	Peripheral outer nuclear layer thinning Later macular involvement	Peripheral hypoautofluroescence, spreading centripetally HyperAF ring may be present	Preserved initially	Progressive visual field constriction	Full field: Rod dysfunction with later cone involvement
CDH3	Macular dystrophy	Childhood	Difficulty reading Blurred central vision Central scotomas	Chorioretinal atrophy at the macula,	Variable atrophy of the outer retina,	Hypoautofluorscence at the macula	Significantly affected	Central scotomas	pERG: Abnormal to undetectable

				contiguous with the disc	RPE and choroid				Full field ERG: normal to mildly subnormal

Gene [UniProt ID]	**Signal peptide**	**Extracellular cadherin domains**	**Transmembrane domain**	**Cytoplasmic domain**	**OVERALL**
CDHR1 Q96JP9-1	Identical sites – 0% Pairwise identity – 38.6% Pairwise positive (BLSM62) – 49.1%	Identical sites – 63.4% Pairwise identity – 84.5% Pairwise positive (BLSM62) – 92.0%	Identical sites – 52.4% Pairwise identity – 81.2% Pairwise positive (BLSM62) – 91.4%	Identical sites – 31.2% Pairwise identity – 63.6% Pairwise positive (BLSM62) – 78.4%	Identical sites – 56.0% Pairwise identity – 79.9% Pairwise positive (BLSM62) – 88.2%
CDH23 Q9H251-1	Identical sites – 17.4% Pairwise identity – 54.0% Pairwise positive (BLSM62) – 63.8%	Identical sites – 73.4% Pairwise identity – 89.9% Pairwise positive (BLSM62) – 94.8%	Identical sites – 90.5% Pairwise identity – 96.8% Pairwise positive (BLSM62) – 98.4%	Identical sites – 69.5% Pairwise identity – 87.5% Pairwise positive (BLSM62) – 92.1%	Identical sites – 72.8% Pairwise identity – 89.5% Pairwise positive (BLSM62) – 94.3%
PCDH15 Q96QU1-1	Identical sites – 19.2% Pairwise identity – 52.0% Pairwise positive (BLSM62) – 64.1%	Identical sites – 72.1% Pairwise identity – 90.2% Pairwise positive (BLSM62) – 94.6%	Identical sites – 71.4% Pairwise identity – 91.8% Pairwise positive (BLSM62) – 97.3%	Identical sites – 23.8% Pairwise identity – 65.9% Pairwise positive (BLSM62) – 73.2%	Identical sites – 59.0% Pairwise identity – 84.0% Pairwise positive (BLSM62) – 89.2%
CDH3 P22223-1	Identical sites – 22.4% Pairwise identity – 54.4% Pairwise positive (BLSM62) – 58.2% Sequences - 6	Identical sites – 56.3% Pairwise identity – 81.1% Pairwise positive (BLSM62) – 89.7%	Identical sites – 17.4% Pairwise identity – 56.3% Pairwise positive (BLSM62) – 63.9%	Identical sites – 76.5% Pairwise identity – 91.7% Pairwise positive (BLSM62) – 96.2%	Identical sites – 54.9% Pairwise identity – 80.2% Pairwise positive (BLSM62) – 87.5%

Supplementary Table 1. Sequence alignment of retinal cadherin protein sequences. Data indicating the degree of homology within extracellular cadherin and cytoplasmic domains, extracted from Homolgene (source: https://www.ncbi.nlm.nih.gov/homologene). Sequence alignments were performed for seven species whose sequences were available for all four cadherins: *H. sapiens, P. troglodyes, C. lupus, B. Taurus, M. musculus, R. norvegicus, G. gallus.*

Supplementary Table 2. Pathological sequence variants in *CDHR1*.

Sequence variants in *CDHR1* for transcript NM_033100.4. Variants predicted to be pathological without published clinical validation were not included. Variants are illustrated against the CDHR1 protein in Figure 4. All listed variants are clinically validated, current at the time of submission. Additional reported pathological sequence variants in the *CDHR1* gene may found using ClinVar, GnomAD and other genetic databases.

***CDHR1* variant**	**Consequence**	**Mean allele frequency dbSNP**	**Phenotype** (where specified for homozygous cases)	**Initial report**
c.1A>G	p.Met1?	0.00002024 rs794726954	-	(Ba-Abbad et al., 2020)
c.18G>A	p.Try6*	0.00001651 rs1220602138	-	(Charbel Issa et al., 2019)
c.56-1G>A	Splice acceptor	0.00001198 rs751972593	RCD	(Stingl et al., 2017)
c.152-2A>G	Splice acceptor	-	CRD	(Bessette et al., 2017)
c.296A>G	p.Glu99Gly	-	CD	(Stingl et al., 2017)
c.297+1G>T	Splice donor	0.000007953 rs765924534	-	(Ba-Abbad et al., 2020)
c.338del	p.Gly113Alafs*2	0.000003979 rs747425652	CRD	(Henderson et al., 2010)
c.386A>G	p.Asn129Ser	0.00001277 rs1424495418	CD	(Oishi *et al.*, 2016)
c.398C>G	p.Pro133Arg	-	-	(Tiwari *et al.*, 2016)
c.438+1G>A	Splice donor	-	-	(Charbel Issa et al., 2019)
c.439-17G>A	Intron	0.000008672 rs771510913	-	(Tiwari et al., 2016)

c.524dup	p.Gln176Glnfs*48	-	CRD	(Ostergaard et al., 2010)
c.562G>A	p.Gly188Ser	0.00006820 rs748412274	-	(Ba-Abbad et al., 2020)
c.639+1del	Splice donor	0.000004173 rs745932099	-	(Stingl et al., 2017)
c.783G>A	p.Pro261=	0.003052 rs147346345	Macular dystrophy	(Charbel Issa et al., 2019, Glöckle *et al.*, 2014)
c.838C>T	p.Arg280*	0.00001988 rs191858417	CRD	(Boulanger-Scemama *et al.*, 2015)
c.928C>T	p.Gln310*	-	-	(Stingl et al., 2017)
c.1311_1316del	p.Leu437_Thr438del	0.000003983 rs1257781536	-	(Stingl et al., 2017)
c.1373T>A	p.Val458Asp	0.000003976 rs760942217	-	(Ba-Abbad et al., 2020)
c.1381C>T	p.Gln461*	-	RCD	(Duncan et al., 2012)
c.1448A>G	p.Glu483Gly	-	-	(Stingl et al., 2017)
c.1463del	p.Gly488fs*20	0.00005966 rs756678484	CRD	(Ba-Abbad et al., 2013) (Henderson et al., 2010)
c.1485+2T>G	Splice donor	0.000003980 rs767366723	CRD	(Cohen et al., 2012)
c.1485+2T>C	Splice donor	0.000007959 rs767366723	RCD	(Nikopoulos et al., 2015)
c.1503_1507del	p.Gly502Leufs*32	0.00001061 rs1266986282	CRD	(Charbel Issa et al., 2019)
c.1527T>G;	p.Tyr509*	0.000003976 rs1477733493	CRD	(Arno *et al.*, 2016)
c.1554-2A>C	Splice acceptor	-	RCD	(Riera *et al.*, 2017)
c.1570_1592del	p.Ser524Alafs*4	0.000003976 rs751597954	CRD	(Charbel Issa et al., 2019)

c.1700T>C	p.Leu567Pro	-	RCD	(Stingl et al., 2017)
c.1720C>G	p.Pro574Ala	-	CRD	(Nikopoulos et al., 2015)
c.2040+5 G>T	Splice donor	0.000003984 rs1446751435	CRD	(Stingl et al., 2017)
c.2108G>A	p.Gly703Asp	-	RCD	(Stingl et al., 2017)
c.2203C>A	p.Pro735Thr	0.000007975 rs780447091	-	(Ba-Abbad et al., 2020)
c.2522_2528del	p.Ile841Serfs119*	0.00003313 rs752341696	RCD	(Arno et al., 2016)

Supplementary Table 3. Pathological sequence variants in *CDH23*. Sequence variants in CDH23 for transcript NM_022124.6. Variant are illustrated against the CDH23 protein in Figure 5. All listed variants are clinically validated, current at the time of submission. Additional reported pathological sequence variants in the *CDH23* gene may found using ClinVar, GnomAD and other genetic databases.

***CDH23* variant**	**Consequence**	**Mean allele frequency (GnomAD)**	**Phenotype**	**Initial report**
c.130G>A	p.Glu44Lys	0.00003184 rs1390133151	USH1	(Okano *et al.*, 2019)
c.172C>T	p.Gln58*	0.000004011 rs758698850	USH1	(Astuto et al., 2002)
c.193del	p.Leu65Trpfs*49	- rs796051861	USH1	
c.336+1G>A	Splice donor	0.00006820 rs764824311	USH1(atypical)	
c.371A>G	p.Asp124Gly	0.000004012 rs751192273	RNSD	
c.719C>T	p.Pro245Leu	0.00004012 rs121908354	RNSD	(Wagatsuma *et al.*, 2007)
c.902G>A	p.Arg305Gly	0.00001069 rs121908355	RNSD	
c.1087del	p.Val363Serfs*20	0.00004412 rs538034348	USH1	(Astuto et al., 2002)
c.1096G>A	p.Ala366Thr	0.007103 rs143282422	USH1	(Ouyang et al, 2005)
c.1355A>G	p.Asn452Ser	rs375646885 0.000008022	RNSD	(Astuto et al., 2002)
c.1439T>A	p.Leu480Gln	rs767928788 0.00003187	RNSD	(Astuto et al., 2002)

p.1450G>C[a]	p.Ala484Pro	-	USH1	(Astuto et al., 2002)
c.1474C>T	p.Gln492*	-		(Bork et al., 2001)
c.1745G>A	p.Arg582Gln	0.00003026 rs200263980	RNSD	(Astuto et al., 2002)
c.2263C>T	p.His755Tyr	0.002019 rs181255269	USH1	(Oshima et al., 2008)
c.2289+1G>A	Splice donor	0.00002039 rs769433759	USH1	(Astuto et al., 2002)
c.2572G>A	p.Val858Ile	0.0003861 rs181275139	USH1	(Zhang et al., 2020)
c.2891G>A	p.Arg964Gln	0.0002168 rs376560330	USH1	
c.IVS23+1G>A	Splice donor		USH1	(Bork et al., 2001)
c.2977G>A	p.Asp993Asn	-	RNSD	(Koohiyan *et al.*, 2020)
c.3105A>C[a]	p.Thr1035Thr	-	USH1	(Astuto et al., 2002)
c.3178C>T	p.Arg1060Trp	0.0004642 rs201536811	RNSD	
c.3268G>A	p.Val1090Ile	0.00002860 rs368487578	USH1	(Oshima et al., 2008)
c.3293A>G	p.Asn1098Ser	0.002209 rs41281310	USH1	(Oshima et al., 2008)
c.3557G>A	p.Gly1186Asp	-	RNSD	(Astuto et al., 2002)
c.3617C>G	p.Pro1206Arg	-	USH1	
c.3625A>G	p.Thr1209Ala	0.01378 rs41281314	USH1	
c.3840insATGA	p.Met1279Ilefs*1		USH1 (atypical)	
c.3718G>A	p.Asp1243Asn	rs975836593 0.000004024	RNSD	(Bork et al., 2001)
c.3913C>T	p.Arg1305*	-	USH1	(Bork et al., 2001)

c.4021G>A	p.Asp1341Asn	0.000004066 rs121908351	RNSD	(de Brouwer *et al.*, 2003)
c.4136G>T	p.Arg1379Leu	-	Pituitary adenoma	(Zhang et al., 2017)
c.4198G>A	p.Asp1400Asn	-	RNSD	(Bork et al., 2001)
c.4488G>C[a]	p.Gln1496His	- rs121908347	USH1	(Bolz et al., 2001)
c.4504C>T	p.Arg1502*	0.00002411 rs769742202	USH1	(Becirovic *et al.*, 2008)
c.4520G>A	p.Arg1507Gln	0.00007843 rs373480195	USH1	(Becirovic et al., 2008)
c.4567A > G	p.Asn1523Asp	0.000008023 rs747906567	RNSD	Liang et al, 2018
c.4756G>C	p.Ala1586Pro	0.000004124 rs573737471	RNSD	(Astuto et al., 2002)
c.4783G>A	p.Glu1595Lys	0.00002079 rs778204574	RNSD	
c.5147A>C	p.Gln1716Pro	- rs758382198	RNSD	(Wagatsuma et al., 2007)
c.5237G>A	p.Arg1746Gln[b]	0.00007127 rs111033270	USH1	(Bolz et al., 2001)
c.5363C>T	p.Pro1788Leu	0.0001053 rs564555435	USH1	(Oshima et al., 2008)
c.5536G>A	p.Asp1846Asn	0.000008019 rs746323558	RNSD	(Astuto et al., 2002)
c.5712G>A	p.Thr1904Thr Skipping of exon 42	0.000008048 rs397517342	USH1	(von Brederlow *et al.*, 2002)
c.5788G>A	p.Asp1930Asn	-	USH1	(Oshima et al., 2008)
c.5821_6253del	p.Tyr1942Serfs*23	-	USH1	(Nakanishi *et al.*, 2010)
c.6049+1G>A	Splice donor	- rs111033247	USH1 (atypical)	(Bork et al., 2001)
c.6050-15G>A	Splice region	0.00001372	USH1 (atypical)	(Valero *et al.*, 2019)

		rs373838930		
c.6050-9G>A	Splice region	0.00004888 rs367928692	USH1	(von Brederlow et al., 2002)
c.6085C>T	p.Arg2029Trp	0.00002445 rs750880909	RNSD	(Wagatsuma et al., 2007)
c.6133G>A	p.Asp2045Asn	0.000004024 rs121908348	RNSD	(Bork et al., 2001)
c.6155del	p.Thr2051Argfs*28	-	USH1 (atypical)	(Astuto et al., 2002)
c.6307G>T	p.Glu2103*	-	USH1	
c.6319C>T	p.Arg2107*	-	USH1	(Bork et al., 2001)
c.6337C>T	p.Gln2113*	0.00001607 rs771210121	USH1	(Oshima et al., 2008)
c.6346_6347del	p.Phe2115Profs*13	-	USH1	(Oshima et al., 2008)
c.6392del	p.Ile2137Serfs*11	0.00001426 rs754876029	USH1	(Oshima et al., 2008)
c.6442G>A	p.Asp2148Asn	0.00009268 rs111033271	RNSD	(de Brouwer et al., 2003)
c.6460C>T	p.Arg2154Cys	-	RNSD	(Bork et al., 2001)
c.6511del	p.Arg2170Alafs*11	-	USH1	(Oshima et al., 2008)
c.6604G>A	p.Asp2202Asn	0.00001071 rs121908349	RNSD	(Bork et al., 2001)
c.6769C>A	p.Pro2257Thr	-		(Bork et al., 2001)
c.6933del	p.Pro2311Profs*62	-	USH1	(von Brederlow et al., 2002)
c.6968del	p.Pro2323Leufs*50	- rs397517350	USH1 (atypical)	(Astuto et al., 2002)
c.IVS51+5G>A	Splice region		USH1	(Bolz et al., 2001)
c.7127A>T	p.Asp2376Val	-	USH1	(Oshima et al., 2008)
c.7393C>T	p.Arg2465Try	0.000008023 rs760879110	RNSD	(Astuto et al., 2002)
c.7549A>G	p.Ser2517Gly	0.00002006 rs759093040	USH1	
c.7589C>T	p.Thr2530Ile	-	USH1	(Oshima et al., 2008)

c.7823G>A	p.Arg2608His	0.0006323 rs202052174	RNSD	(Astuto et al., 2002)
c.7872G>A[a]	p.Glu2624Glu	0.000004964 rs1292050472	USH1	
c.8230G>A	p.Gly2744Ser	0.00004830 rs376189742	USH1 (atypical)	
c.8311G>A	p.Gly2771Ser	0.0003761 rs201076440	USH1	(Oshima et al., 2008)
c.8497C>G	p.Arg2833Gly	0.000004074 rs760130862	USH1 (atypical)	(Astuto et al., 2002)
c.8849T>A	p.Ile2950Asn	-	RNSD	(Bork et al., 2001)
c.8722+1del	Splice donor	-	USH1	(Oshima et al., 2008)
c.8866C>T	p.Arg2956Cys	0.00001795 rs751367894	RNSD	(Bork et al., 2001)
c.8903T>C	p.Val2968Ala	0.000008098 rs765847991	USH1	(Oshima et al., 2008)
c.9175C>A	p.Pro3059Thr	0.00002813 rs780514498	RNSD	(Bork et al., 2001)
c.IVS66+1G>A	Splice donor	-	USH1 (atypical)	
c.9319+1G>T	Splice donor	-	USH1	(Menghini et al., 2019)
c.9524G>A	p.Arg3175His	0.0002622 rs140884994	USH1	(Astuto et al., 2002)
c.9626ins	p.Pro3208Profs*37	-	USH1	

a – predicted to reduce efficiency of splicing; RNSD – recessive non-syndromic deafness; [b] -missense variant shown to affect splicing in minigene assay(Becirovic et al., 2008).

Supplementary Table 4. Pathological sequence variants in *PCDH15*. Sequence variants in *PCDH15* for transcript NM_033056.3. Variants are illustrated against the PCDH15 protein in Figure 6. All listed variants are clinically validated, current at the time of submission. Additional reported pathological sequence variants in the *PCDH15* gene may found using ClinVar, GnomAD and other genetic databases.

***PCDH15* variant**	**Protein consequence**	**Mean allele frequency (GnomAD)**	**Phenotype**	**Initial report**
c.7C>T	p.Arg3*	0.00002013 rs137853001	USH1	(Ahmed et al., 2001)
c.16del	p.Tyr6Ilefs*5	- rs397517451	USH1	(Ouyang *et al.*, 2005) (Zheng et al., 2005)
c.158-1G>A	Splice acceptor	- rs876657418	USH1	(Yoshimura *et al.*, 2014)
c.400C>G	p.Arg134Gly	-	RNSD	(Ahmed et al., 2003)
c.401G>A	p.Arg134Gln	0.00001063 rs767966376	USH1	(Jaijo et al., 2012)
c.533A>G	p.Asp178Gly	-	USH1	(Ahmed et al., 2008)
c.536A>G	p.Asn179Ser	- rs145037203	USH1	(Jaijo et al., 2012)
c.733C>T	p.Arg245*	0.0002018 rs111033260	USH1	(Ben-Yosef et al., 2003)
c.785G>A	p.Gly262Asp	- rs137853002	RNSD	(Ahmed et al., 2003)
c.996-999del	p.Glu332Aspfs*20	-	USH1	(Ouyang et al., 2005)
c.1088del	p.Leu363Trpfs*58	- rs199469706	USH1	(Alagramam et al., 2001b)

c.1304A>C	p.Asp435Ala	0.2327 rs4935502	USH1	(Saleha et al., 2016)
c.1304_1305insC	p.Thr436Tyrfs*12		USH1	(Jaijo et al., 2012)
c.1583 T>A	p.Val528Asp	0.000003979 rs267606932	RNSD	(Doucette *et al.*, 2009)
c.1737C>G	p.Tyr579*	0.000003985 rs1057517251	USH1	(Jaijo et al., 2012)
c.1940C>G	p.Ser647*	- rs137853004	USH1	(Ahmed et al., 2008)
c.2052C>A	p.Tyr684*	-	USH1	(Ahmed et al., 2008)
IVS27-2A>G	Splice acceptor	-	USH1	(Ahmed et al., 2001)
c.2782A>T	p.Lys928*	-	USH1	(Jaijo et al., 2012)
c.2785C>T	p.Arg929*	0.000007974 rs1057516342	USH1	(Ouyang et al., 2005)
c.2868+5G>A	Splice region	0.000003987 rs757993503	USH1	(Jaijo et al., 2012)
c.3717+1G>T	Splice donor	0.000007088 rs748706627	USH1	(Ahmed et al., 2008)
c.3717+2dup	Splice donor	0.00001196 rs1248401224	USH1	(Jaijo et al., 2012)
c.4024C>A	p.Gln1342Lys	0.002210 rs61731387	USH1	(Ouyang et al., 2005)
c.4257del	p.Leu1419Phefs*99	-	USH1	(Ahmed et al., 2008)
c.5603_5605del	p.Thr1876del	0.009159 rs747596359	USH1	(Ouyang et al., 2005)

Supplementary Table 5. Pathological sequence variants in *CDH3*. Sequence variants in CDH3 for transcript NM_001793.6. Variants are illustrated against the CDH3 protein in Figure 7. All listed variants are clinically validated, current at the time of submission. Additional reported pathological sequence variants in the *CDH3* gene may found using ClinVar, GnomAD and other genetic databases.

***CDH3* variant**	**Protein consequence**	**Mean allele frequency (GnomAD)**	**Phenotype**	**Initial report**
c.160+1G>A	Splice donor	0.00001394 rs1474181679	HJMD/EEMS	(Indelman *et al.*, 2007)
c.del161−811_246+1,044	Alu recombination-mediated deletion	-	HJMD	(Saeidian *et al.*, 2019)
c.307G>T	p.Arg103*		HJMD	(Khan and Bolz, 2016)
c.316_317del	p.Lys106Glufs*12	-	EEMS	(Hull et al., 2016)
c.353A>G	p.Glu118Gly	-	EEMS	(Shimomura *et al.*, 2008)
c.447_467del	p.149_156del	-	HJMD	(Karti *et al.*, 2017)
c.462del	p.Glu155Argfs*6	0.00001415 rs749189312	HJMD	(Indelman *et al.*, 2003)
c.490dup	p.Thr164Asnfs*8	0.000003977 rs757142746	HJMD	(Shimomura et al., 2008)
c.503T>A	p.Leu168*	0.000003977 rs765438205	HJMD	(Indelman et al., 2003)
c.613G>A	p.Val205Met	-	HJMD	(Hull et al., 2016)

c.640A>T	p.Lys214*	-	HJMD	(Almeida *et al.*, 2018)
c.661C>T	p.Arg221*	0.000007968 rs761941770	HJMD	(Indelman et al., 2007)
c.747C>A	p.Tyr249*	-	HJMD	(Avitan-Hersh *et al.*, 2012)
c.830del	p.Gly277Alafs*20	- rs724159985	HJMD EEMS	(Indelman et al., 2003, Kjaer et al., 2005)
c.830G>T	p.Gly277Val	-	EEMS	(Basel-Vanagaite et al., 2010)
c.965A>T	p.Asn322Ile	- rs121434543	EEMS	(Kjaer et al., 2005)
c.981del	p.Met327fs*23	- rs724159984	HJMD	(Sprecher et al., 2001)
c.1086G>A	p.Trp362*	0.000007075 rs779888126	EEMS	(Hull et al., 2016)
c.1425-1G>A	Splice	-	HJMD	(Kamran-ul-Hassan Naqvi *et al.*, 2010)
c.1425-1G>T	Splice	0.000007958 rs757246377	HJMD	(Shimomura et al., 2008)
c.1508G>A	p.Arg503His	0.000003977 rs121434542	HJMD	(Indelman et al., 2002)

c.1510G>A	p.Glu504Lys	-	EEMS	(Indelman et al., 2007)
c.1568del	p.Asn523Metfs*14	-	HJMD	(Hull et al., 2016)
c.1571-2A>G	Splice acceptor	-	Scalp hypotrichosis only	(Shimomura et al., 2008)
Deletion of 8,815bp	In-frame deletion	-	HJMD	(Halford *et al.*, 2012)
c.1724A>G	p.His575Arg	0.00003189 rs1310647677	HJMD	(Indelman et al., 2007)
c.1796-2A>G	Splice acceptor	0.00001988 rs757728994	HJMD	(Shimomura *et al.*, 2010)
c.1845T>A	p.Tyr615*	-	HJMD	(Indelman *et al.*, 2005)
c.1859_1862del	p.Ser619Cysfs*10	-	MD without hypotrichosis	(Khan and Bolz, 2016)
c.2117del	p.Gly706Glufs*10	-	HJMD	(Indelman et al., 2003)
c.2157C>T	p.Arg720*	0.000007073 rs368064604	HJMD	(Hull et al., 2016)
c.2357del	p.Gly786Alafs*7	-	HJMD	(Hull et al., 2016)

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