The X-linked retinopathies: Physiological insights, pathogenic mechanisms, phenotypic features and novel therapies

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ABSTRACT

X-linked retinopathies represent a significant proportion of monogenic retinal disease. They include progressive and stationary conditions, with and without syndromic features. Many are X-linked recessive, but several exhibit a phenotype in female carriers, which can help establish diagnosis and yield insights into disease mechanisms. The presence of affected carriers can misleadingly suggest autosomal dominant inheritance. Some disorders (such as RPGR-associated retinopathy) show diverse phenotypes from variants in the same gene and also highlight limitations of current genetic sequencing methods. X-linked disease frequently arises from loss of function, implying potential for benefit from gene replacement strategies.

We review X-inactivation and X-linked inheritance, and explore burden of disease attributable to X-linked genes in our clinically and genetically characterised retinal disease cohort, finding correlation between gene transcript length and numbers of families. We list relevant genes and discuss key clinical features, disease mechanisms, carrier phenotypes and novel experimental therapies. We consider in detail the following: RPGR (associated with retinitis pigmentosa, cone and cone-rod dystrophy), RP2 (retinitis pigmentosa), CHM (chori-deremia), RS1 (X-linked retinoschisis), NYX (complete congenital stationary night blindness (CSNB)), CACNA1F (incomplete CSNB), OPN1LW/OPN1MW (blue cone monochromacy, Bornholm eye disease, cone dystrophy), GPR143 (ocular albinism), COL4A5 (Alport syndrome), and NDP (Norrie disease and X-linked familial exudative vitreoretinopathy (FEVR)). We use a recently published transcriptome analysis to explore expression by cell-type and discuss insights from electrophysiology. In the final section, we present an algorithm for genes to consider in diagnosing males with non-syndromic X-linked retinopathy, summarise current experimental therapeutic approaches, and consider questions for future research.

1. Introduction

Inherited retinal diseases are a frequent cause of blindness in pediatric and working age populations in many countries (Liew et al., 2014; Rahman et al., 2020; Solebo and Rahi, 2014; Solebo et al., 2017).

The X-linked retinopathies represent an important, diverse subgroup, including both progressive and stationary conditions, and disorders with and without syndromic features. They display a number of different pathogenic mechanisms, thereby yielding important and wide-ranging insights into key aspects of retinal physiology and pathophysiology. A significant number are also the subject of novel therapeutic trials.
Pathogenic variants usually cause disease by loss of protein function; hence these conditions are particularly attractive for gene-replacement strategies. There is a carrier phenotype in several of these conditions, which can help clinicians narrow the differential diagnosis and thereby mode of inheritance, and which may also reveal valuable information regarding patterns of X inactivation, normal retinal cellular development and migration, and mechanisms of disease. Carrier females are often asymptomatic, but may infrequently be as severely affected as males, which may result in inheritance being incorrectly classified as autosomal dominant. Some of the disorders also illustrate ways in which variants in the same gene can generate a number of phenotypes, and potential limitations in current genetic sequencing methods; awareness of the latter is particularly relevant as we enter the era of widely available whole genome sequencing and gene therapy.

In this article, X-inactivation and patterns of X-linked inheritance will be reviewed briefly. We then explore the burden of disease attributable to X-linked genes in a large clinically and genetically characterised inherited retinal disease cohort, highlighting the most frequently implicated genes, and investigating correlation between gene transcript length and numbers of affected families. Subsequently, we provide a list of X-linked genes involved in a number of non-syndromic and syndromic retinopathies, together with key clinical features, and a schematic showing sites of expression or impairment in the retina. We then discuss several genes in more detail as specified in the abstract. For several conditions, disease mechanisms, clinical features, carrier phenotypes, electrophysiological findings, and novel experimental therapies, where applicable, are considered. A comprehensive, detailed discussion of molecular mechanisms for each condition, however, is outside the scope of this review. Exemplary findings on multimodal retinal imaging (including ultra-widefield fundus imaging, fundus autofluorescence (FAF) and spectral domain optical coherence tomography (OCT)) are included for a number of these disorders, which will be of use as an image library to clinicians managing affected patients.

In the following section, findings from a recent single-cell transcriptome analysis are used to investigate patterns of expression by cell-type for genes associated with X-linked retinopathy. In vivo electrophysiology in patients is also discussed together with insights into the processes shaping the bright-flash electroretinogram (ERG) a-wave peak yielded by a consideration of recordings in complete CSNB. A selection of ERG parameters from a cohort of X-linked retinitis pigmentosa (XLRP) carriers is also presented.

The final section highlights some key messages, presents an algorithm for deciding which genes to consider when investigating a male with a non-syndromic X-linked retinopathy, and summarises current experimental approaches to therapy as well as considering questions for future research.

2. The X chromosome, X-linked inheritance and X-inactivation

The human sex chromosomes, X and Y, are evolved from autosomes with the first evolutionary events occurring between 240 and 320 million years ago (Lahn and Page, 1999). It is believed that chromosomal inversions disrupted recombination of these autosomes during meiosis, resulting in formation of heteromorphic sex chromosomes, with subsequent degeneration of the Y chromosome (Charlesworth and Charlesworth, 2000). Females inherit one X chromosome from each parent (XX), whereas males inherit an X chromosome from their mother and Y chromosome from their father (XY). Diseases caused by sequence variants in genes on the X chromosome are known as X-linked, and due to this chromosomal inheritance pattern, male to male transmission is never seen in a pedigree for X-linked disease (Fig. 1A). Some female carriers may however manifest features of X-linked recessive disease leading to apparent pseudo-dominant inheritance (Fig. 1B). This led historically to the conclusion that X-linked inheritance was rare in retinal dystrophies (Francois, 1961), which was subsequently demonstrated to be erroneous, with X-linked inheritance being demonstrated in up to a fifth of families with RP (Bird, 1975; Jay, 1982). Such errors are still being corrected: in a recent publication, we showed that disease in a family previously published as autosomal dominant (Downes et al., 2001) was in fact likely to be due to a pathogenic variant in the X-linked RPRG gene, and the autosomal variant was likely to be benign (Mahroo et al., 2019).

The X chromosome encodes around 1000 genes, compared with approximately 70 genes on the Y chromosome, and the presence of two X chromosomes in females generates the need for mechanisms to equalise dosage of X chromosome genes in females to that seen in males. This is achieved by X-inactivation whereby either the maternal or paternal X chromosome is transcriptionally silenced early in embryogenesis (Lyon, 1961), with transmission of this choice to all clonally derived cells via mitosis. This ‘random’ form of X-inactivation occurs in placental mammals and is a complex process that is not entirely understood. Cellular mechanisms detect the presence of more than one X chromosome and activate an X-inactivation pathway. There are several interacting components including the X (inactive)-specific transcript (Xist) which initiates silencing of the future inactive X chromosome and its antisense partner Tsix and the intergenic locus Xite which preserve the transcriptional ability of the future active X chromosome (Huynh and Lee, 2005).

X-inactivation can be demonstrated in the retina by clonal analysis of retinal progenitor cells. Using transgenic female mice with labelled X chromosomes, retinal progenitor cells arising from inactivation of each X chromosome can be identified depending on cell labelling (Reese and Tan, 1998) (Wu et al., 2014). Columns of labelled or unlabelled cells are evident in the developing retina indicating inactivation of either X chromosome, as well as radial migration of columns of labelled retinal neuroblasts. The latter might explain the pattern of pigmentation seen in
carriers of some X-linked retinal diseases such as ocular albinism or choroideremia.

However, not all genes undergo X-inactivation, with around 15% of genes on the X chromosome escaping inactivation in humans, i.e. being expressed by both X chromosomes (Carrel and Willard, 2005), and a further 10% demonstrating variable patterns of inactivation. A review of X-linked genes known to cause retinal disease demonstrated that RPGR, RP2, and CACNA1F all undergo complete X-inactivation, RS1 demonstrates variable escape, and others known to cause X-linked retinal disease are undetermined (Fahim and Daiger, 2016). The clinical significance of this is unclear.

X-inactivation may also be skewed in that there is preference for either the maternal or paternal X chromosome to be inactivated. This may be a result of a chromosomal abnormality leading to preferential inactivation, or a stochastic event. There is evidence that skewed inactivation may also increase with age in certain tissues especially blood (Hatakeyama et al., 2004) but there are few data to correlate this with changes in the retina. Skewed X-inactivation is hypothesised to be responsible for female carriers manifesting signs of X-linked disease, which can be of variable severity in different individuals. Evidence to support this has recently been shown by examining X chromosome inactivation ratios in blood and saliva in RPGR carriers, in whom increased skewing towards inactivation of the normal allele was associated with worse visual function (Fahim et al., 2020).

3. Prevalence of X-linked disease and associated genes in a large genotyped inherited retinal disease cohort

The inherited retinal disease service at Moorfields Eye Hospital in London is the biggest single center which manages patients with monogenic retinal conditions in the United Kingdom. Pedigrees in whom a molecular cause has been found (by genetic screening methods including single gene tests, next generation sequencing panels, whole exome and whole genome sequencing) and judged by the clinical team to be in keeping with the phenotype and mode of inheritance, are entered with a unique identifier into the electronic patient record. We recently conducted a retrospective search of this record (search date August 2019), which yielded over 3000 families with a molecular diagnosis. For this study, those genes listed on the Retinal Information Network’s online “RetNet” resource (https://sph.uth.edu/retnet/) were included, as well as, for the analysis below, GPR143 (in which pathogenic variants lead to X-linked ocular albinism). The search was also repeated including only patients aged under 18 years to facilitate assessment of the proportion of paediatric inherited retinal disease attributable to each gene. The broad results of this investigation have been recently published (Pontikos et al., 2020), but here the findings in relation to X-linked disease are examined in more detail.

Fig. 2 shows the prevalence of X-linked disease in these cohorts (Fig. 2A). In the overall cohort (3199 families), 13.8% of families had disease associated with variants in X-linked genes. In the paediatric cohort (412 families) the proportion was greater (20.9%). This might reflect both the earlier onset and severity of some X-linked conditions.
(compared with autosomal and mitochondrial retinopathies) and also that diagnoses are likely to be reached earlier in the presence of a positive family history in which both families and physicians may be alerted to the possibility of the disease. Recently published data from the Center for Hereditary Retinal Degenerations at the University of Pennsylvania found a similar proportion in their patients: of 1656 patients who underwent genetic testing, the genetic basis was found in 52%, and of these, 17% had X-linked disease (Garafalo et al., 2019).

The proportions of families with X-linked disease attributable to each gene in the Moorfields cohorts are depicted in Fig. 2B: in both cohorts, the same genes are seen, but the relative frequencies differ. The number of affected individuals by gene is depicted in Fig. 2C. As expected the overwhelming majority are males, but for three genes, RPGR, RP2 and CHM, affected females are also seen. Female carriers of pathogenic
variants in RPGR can be symptomatic. In choroideremia, females can develop symptoms, but this tends to be later in life (likely explaining the absence of females in the paediatric cohort).

Some limitations of this analysis merit consideration. This was a retrospective search over a time period (dating back to 2003) during which criteria for genetic testing as well as methods of sequencing have evolved considerably. Genes that were discovered earlier, or which were subjects of particular clinical or research interest (including those that were candidates for novel therapeutic trials), or which were more amenable to sequencing by earlier methods, are likely to be over-represented. More recent testing was less biased, making use of large gene panels as well as whole exome, and whole genome sequencing (as part of national collaborative projects (Carss et al., 2017; Turnbull et al., 2018)). Also, this analysis does not necessarily indicate the proportion of all suspected genetic disease that is X-linked. Even in the era of whole genome sequencing, over 30% of patients with suspected inherited retinal disease still do not achieve a molecular genetic diagnosis; a previous study showed that only 63% of patients with no prior testing achieved a molecular diagnosis from whole exome or whole genome sequencing (Carss et al., 2017). Of those patients with monogenic disease in whom the cause has not been found, Garafalo et al. (2019) reported that 4% had an X-linked mode of inheritance.

Patients with disease caused by variants in the opsin array (OPN1LW/OPN1MW) might be under-represented in our cohort since these variants are not easily detected by standard sequencing methods, including whole genome sequencing, and have been identified via research programmes both internally and externally for patients with clinical features of these conditions. Also, as our service is part of a stand-alone eye hospital, genes implicated in syndromic retinopathies, in which specialist multi-disciplinary medical care is required (particularly those in which non-ocular features may be more prominent), are likely to be under-represented. Nevertheless, our cohort is the largest molecularly characterised monogenic retinal disease cohort to date, and the analysis affords a quantitative appreciation of the burden of disease attributable to the various X-linked genes.

Fig. 3 plots the number of families against transcript length for each gene. Gene transcript length is of relevance in the development of gene-replacement therapies, with smaller transcripts being more amenable to packaging within adeno-associated viral vectors. A significant positive correlation was apparent (though the number of genes was small): genes with longer transcripts were represented by more families in both the overall cohort and the paediatric cohort (depicted in upper and lower panels respectively; correlation coefficients are given in the Figure legend). Longer genes might be expected to have a greater probability of harbouring pathogenic variants such as a premature termination (given that loss of function is a key mechanism in X-linked disease), and this might explain the observed relationship. In contrast, for autosomal dominant disease, where disease is frequently attributable to specific gain of function variants, such a relationship might not be expected, and indeed we did not observe a significant positive correlation for autosomal dominant disease (Spearman correlation coefficient, $-0.17; p = 0.46$).

4. X-linked genes and key clinical features

Table 1 lists X-linked genes in which pathogenic variants give rise to a retinopathy with or without syndromic features, together with key clinical features in affected males (except PRPS1 and X-linked dominant disorders). Fig. 4 depicts, in schematic form, the cellular or subcellular location of the expressed protein or associated impairment.

A number of these disorders are discussed in further detail in turn from Section 4.1 onwards. Typical retinal findings on multimodal retinal imaging are also presented (Figs. 5–22). Pseudocolour images were obtained using an ultra-widefield imaging device (Optos plc, Dunfermline, UK), with the same device used for ultra-widefield autofluorescence images (excitation wavelength 532 nm). Spectral domain optical coherence tomography (OCT) images were obtained with the Spectralis device (Heidelberg Engineering, Heidelberg, Germany). The same device was used to obtain short wavelength (488 nm) autofluorescence images and near infrared reflectance images (the latter shown in Fig. 21). Fig. 22 additionally displays images obtained with a white light fundus camera (Fig. 22B) and the RetCam (Massie Research Laboratories, Inc., Dublin, California) device (Fig. 22E and F). These images show characteristic patterns useful for clinicians; readers unfamiliar with these imaging techniques are referred to recent relevant reviews (Nagiel et al., 2016; Yung et al., 2016). In addition, characteristic findings from visual electrophysiology are also discussed (with a summary in Section 6), and readers unfamiliar with these tests are referred to a recent review of diagnostic electrophysiological procedures (Robson et al., 2018).

4.1. RPGR (retinitis pigmentosa and cone or cone-rod dystrophy)

4.1.1. Disease mechanism

The retinitis pigmentosa GTPase regulator (RPGR) gene was the first gene causing X-linked retinitis pigmentosa to be identified (Meindl et al., 1996). RPGR encodes a protein comprising 19 exons, however there are over 20 isoforms of RPGR that arise as a result of alternative splicing (He et al., 2008; Hong and Li, 2002). The full length or constitutive protein translated from all 19 exons, and the key open reading frame 15 (ORF15) variant are the most common. The latter contains exons 1–14 and ORF 15: a 3' region containing exon 15 and a part of intron 15, which encompasses a highly repetitive domain rich in glutamine and
Table 1
X-linked genes associated with syndromic and non-syndromic retinopathies together with chromosomal location, encoded protein and phenotypic features. Some of the genes traditionally associated with disorders considered stationary have in some cases been demonstrated to be associated with progression of degeneration.

| Gene         | Cytogenetic location | Protein                                      | Phenotype/syndrome                        | Retinopathy progressive or stationary | Clinical features                                                                 |
|--------------|----------------------|----------------------------------------------|-------------------------------------------|---------------------------------------|-----------------------------------------------------------------------------------|
| **Conditions with predominantly ocular features** |
| RPGR         | Xp11.4               | Retinitis pigmentosa GTPase regulator        | Rod-cone dystrophy,                      | Progressive                           | Night blindness, visual field (VF) constriction, central visual loss; myopia. Systemic (rare): hearing loss, sinusitis, asthma, chronic respiratory infections, infertility |
|              |                      |                                              | Cone dystrophy, Cone-rod dytrophopy      | Progressive                           | Reduced central vision, photophobia, reduced colour vision, with possible subsequent night blindness and VF constriction following rod system involvement; myopia |
| RP2          | Xp11.3               | RP2 protein, ARL3 GTPase activating protein  | Rod-cone dystrophy                       | Progressive                           | Night blindness, visual field constriction, macular atrophy (earlier than in RPGR), reduction of visual acuity, myopia |
| CHM          | Xq21.2               | Rab escort protein 1 (REP1)                  | Choroideremia                             | Progressive                           | Night blindness, visual field constriction, characteristic retinal degeneration |
| RS1          | Xp22.13              | Retinoschisin                                | X-linked retinoschisis                   | Progressive                           | Reduced visual acuity (often associated with anisometropia and/or strabismus), macular schisis, macular atrophy (later in disease), retinal sheen, peripheral retinoschisis |
| NYX          | Xp11.4               | Nycetalopin                                  | Complete congenital stationary night blindness | Stationary                           | Reduced visual acuity, myopia, night blindness, nystagmus and strabismus |
| CACNA1F      | Xp11.23              | Calcium voltage-gated channel subunit alpha1 F | Incomplete congenital stationary night blindness | Stationary                           | Reduced visual acuity, myopia, night blindness, light sensitivity, nystagmus and strabismus |
| OPN1L1W/OPN1MW | Xq28               | l. and M opsin                               | Blue cone monochromatism,                | Stationary                           | Reduced visual acuity and colour vision, myopia, nystagmus, photophobia. May develop atrophy later in life |
|              |                      |                                              | Cone dystrophy/Cone-rod dystrophy        | Progressive                           | Reduced central vision, photophobia, reduced colour vision, with possible subsequent night blindness and VF constriction following rod system involvement, myopia |
| GPR143       | Xp22.2               | G protein-coupled receptor 143              | Ocular albinism                           | Stationary                           | Nystagmus, refractive error, reduced visual acuity, iris transillumination, albinotic fundus, foveal hypoplasia, abnormal decussation at optic chiasm |
| **Conditions with both ocular and systemic features** |
| COL4A5       | Xq22.3               | Collagen type IV alpha 5 chain               | Alport syndrome                           | May be progressive                    | Ocular: Anterior lenticonus, fleck retina, temporal retinal thinning with hyper-reflective internal limiting membrane; more rarely, macular holes and giant retinal tears |
|              |                      |                                              |                                          |                                      | Systemic: sensorineural deafness, kidney failure |
| NDP          | Xp11.3               | Norrin                                       | Norrie disease,                           | Progressive                           | Ocular: Retinal dysplasia/pseudoglioma, retinal detachment, leucocoria, vitreous haemorrhage, cataract, shallow anterior chamber, corneal opacification, phthisis Systemic: developmental delay, deafness |
|              |                      |                                              | Familial exudative vitreoretinopathy     | May be progressive                    | Ocular: Peripheral temporal retinal avascular zone ± retinal folds, dragged macula, fibrous bands |
| IKBKG/NEMO   | Xq28                 | Inhibitor of nuclear factor koppa B kinase subunit gamma; NF-koppa-B essential modulator | Incontinentia pigmenti                    | May be progressive                    | Ocular: Abnormal peripheral retinal vasculature, glosis, tractional retinal detachment Systemic: Abnormal teeth, cutaneous abnormalities, CNS anomalies |
| Not yet identified | Xp22             |                                              | Aicardi syndrome                         | Stationary/ dysgenesis                | Ocular: Microphthalmia, chorioretinal lacunar defects, colobomas Systemic: agenesis of corpus collosum, infantile spasms, vertebral and rib malformations |
| PRPS1        | Xq22.3               | Phosphoribosyl pyrophosphate synthetase 1    | Photoreceptor dystrophy                  | Progressive                           | Ocular: Patchy mid-peripheral atrophy with interocular asymmetry, macular atrophy in some patients, optic disc atrophy Systemic: Arts syndrome, Charcot–Marie–Tooth, and non-syndromic sensorineural deafness |
| ODF1         | Xq22.2               | ODF1 Centriole and centriolar satellite protein | Rod-cone dystrophy                       | Progressive                           | Ocular: Severe retinal degeneration Systemic: orofaciocutaneous syndrome-1, Jouverture syndrome |
| GLA          | Xq22.1               | Alpha galactosidase A                        | Fabry disease                            | Stationary                           | Ocular: Corneal verticillata, cataract (spoke-like pattern at posterior capsule), conjunctival and retinal vessel tortuosity Systemic: Angiokeratomas, hypohidrosis, painful peripheral neuropathy, tinnitus and hearing loss, renal disease, cardiac disease and stroke |
| ATP7A        | Xq21.1               | ATPase copper transporting alpha             | Menkes disease                           | Unclear, significantly                | Ocular: Myopia, strabismus, iris stromal hypoplasia, optic atrophy, retinal degeneration Systemic: wiry hair, ataxia, neurodegeneration |

(continued on next page)
glycine residues that is a mutational hotspot. Variants in this region are responsible for 60% of cases of X-linked RP (Vervoort et al., 2000). In murine models, the ratio of expressed \( \text{RPGR} \) isoforms has been shown to be critical to photoreceptor integrity, and overexpression of the full-length isoform resulted in photoreceptor degeneration (Wright et al., 2011).

\( \text{RPGR} \) is expressed in several tissues including lung, kidney, testis and brain. In particular, the ORF15 variant localizes to photoreceptor connecting cilia (Hong et al., 2003), where it interacts with other proteins including those encoded by \( \text{RPGRIP1} \) (Mavlyutov et al., 2002), \( \text{PDE6D} \) (Linari et al., 1999) and \( \text{CEP290} \) (Anand and Khanna, 2012). Disruption of this interaction is a common feature of disease-causing \( \text{RPGR} \) missense variants (Zhang et al., 2019). With regard to \( \text{RPGR} \) function, exons 3 to 10 form a regulator of chromosome condensation 1 (RCC1)-like domain and this region may have a role interacting with GTPases (Meindl et al., 1996) such as \( \text{RAB8A} \) (Murga-Zamalloa et al., 2010). The location of \( \text{RPGR} \) at the cilium and its protein associations suggest a key role in ciliary function and protein trafficking or sorting (Megaw et al., 2015), although details of these mechanisms need to be further elucidated.

### 4.1.2. Clinical features

Variants in \( \text{RPGR} \) are responsible for several disease patterns including rod-cone dystrophy (70%), cone-rod dystrophy (6–23%), and cone dystrophy (7%) (Sharon et al., 2003; Talib et al., 2019); with a minority of patients also having extra-ocular disease (Table 1). In general, variants in exons 1–14 and the proximal part of ORF15 are associated with a rod-cone phenotype, and cone/cone-rod dystrophies are predominantly caused by sequence variants towards the 3’ end of the ORF 15 region of \( \text{RPGR} \) (Ebenezer et al., 2005; Talib et al., 2019). Fig. 5 illustrates the phenotype of patients with ORF15 variants from our Moorfields cohort, which clearly demonstrates this effect.

| Gene | Cytogenetic location | Protein | Phenotype/syndrome | Retinopathy progressive or stationary | Clinical features |
|------|---------------------|---------|-------------------|-------------------------------------|------------------|
| LAMP2 | Xq24 | Lysozyme-associated membrane protein 2 | Danon disease (cone-rod dystrophy, some cases) | Progressive | Ocular: Peripheral pigmentary retinopathy Systemic: Cardiomyopathy, myopathy, developmental delay |
| IDS | Xq28 | Iduronate 2-sulfatase | Mucopolysaccharidosis II, Hunter syndrome | Progressive | Ocular: Pigmentary retinopathy, fundus hypopigmented spots, optic nerve head oedema or atrophy, hyperopia, visual field loss Systemic: multisystem disorder due to accumulation of glycosaminoglycans, including airway obstruction, skeletal abnormalities, cardiomyopathy and neurological abnormalities. Ocular: Coloboma of iris, retina or choroid; microphthalmia Systemic: Patchy hypoplastic skin, patchy alopecia, nail abnormalities, split hand/foot deformities |
| PORCN | Xp11.23 | Porcupine O-acyltransferase | Goltz syndrome/Focal dermal hypoplasia | Stationary/dysgenesis | |

Table 1 (continued)

**Fig. 4. Schematic of retina showing main neuronal cell types and location of relevant proteins or sites of impairment.** BM, Bruch membrane; RPE, retinal pigment epithelium; BC, bipolar cell; HC, horizontal cell; AC, amacrine cell; RGC, retinal ganglion cell; ILM, inner limiting membrane.
However, patients with RP may also have variants in a similar region of RPGR as those with cone-rod dystrophy (illustrated by the ‘watershed zone’ depicted in Fig. 5), and both phenotypes have been documented within the same family (Ruddle et al., 2009). The cause for this variation given the same disease-causing variant is unclear, but could feasibly be due to modification of interaction with other proteins. For example, variants in TTLL5 result in a similar cone-rod dystrophy to patients with variants in RPGR ORF15, and TTLL5 variants are known to cause disease by affecting RPGR ORF15 glutamylation (Sergouniotis et al., 2014; Sun et al., 2016).

RPGRIP1, CEP290 and PDE6D interact with RPGR and vary between rod and cone photoreceptors. Other genetic modifiers and environmental factors may have an influence.

4.1.2.1. Retinitis pigmentosa/rod-cone dystrophy. Retinitis pigmentosa (RP) shows X-linked inheritance in 8–16% of patients (Birtel et al., 2018b; Bunker et al., 1984) with the prevalence of affected males being approximately 1:15,000 to 1:26,000 (Prokisch et al., 2007). The RPGR gene is responsible for over 70% of these cases (Sharon et al., 2003). X-linked RP tends to have a more severe phenotype when compared to other causes of RP with autosomal inheritance. Presentation is often in childhood, one study documenting age 5 (range 0–14) as the median age for onset of symptoms, with the first reported symptoms being nyctalopia and peripheral visual loss (Talib et al., 2019). Deterioration in visual acuity in patients with RPGR-associated RP is faster in comparison to RP caused by variants in other genes, reported at around 4–5% per year (Sandberg et al., 2007) (Tee et al., 2018b), which is attributed to a greater rate of foveal thinning and outer nuclear layer loss. The majority of patients eventually progress to legal blindness, although studies report varying timescales ranging from 20% of patients being legally blind by the age of 40 years (Talib et al., 2019) to legal blindness at a median age of 45 (Sandberg et al., 2007). This variability is also influenced by the definition of legal blindness used. Myopia is a very common feature, and best-corrected visual acuity decline has been reported in one study to be greater in high myopes (>6 dioptres) versus those with less refractive error (7.9% versus 3.1% loss of visual acuity per year) (Talib et al., 2019).

There are conflicting reports regarding correlation of the location of RPGR variants and disease phenotype. Some studies report worse visual function in patients with variants in exons 1–14 compared to in ORF15 (Fahim et al., 2011; Sharon et al., 2003; Yang et al., 2014b), whereas others report the opposite (Andreasson et al., 2003; Talib et al., 2019). However there may be a number of factors to consider in interpreting these data, including the phenotypic variability evident in patients in the same family with the same variant, documented variation in the

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4.1.2.1. Retinitis pigmentosa/rod-cone dystrophy. Retinitis pigmentosa (RP) shows X-linked inheritance in 8–16% of patients (Birtel et al., 2018b; Bunker et al., 1984) with the prevalence of affected males being approximately 1:15,000 to 1:26,000 (Prokisch et al., 2007). The RPGR gene is responsible for over 70% of these cases (Sharon et al., 2003). X-linked RP tends to have a more severe phenotype when compared to other causes of RP with autosomal inheritance. Presentation is often in childhood, one study documenting age 5 (range 0–14) as the median age for onset of symptoms, with the first reported symptoms being nyctalopia and peripheral visual loss (Talib et al., 2019). Deterioration in visual acuity in patients with RPGR-associated RP is faster in comparison to RP caused by variants in other genes, reported at around 4–5% per year (Sandberg et al., 2007) (Tee et al., 2018b), which is attributed to a greater rate of foveal thinning and outer nuclear layer loss. The majority of patients eventually progress to legal blindness, although studies report varying timescales ranging from 20% of patients being legally blind by the age of 40 years (Talib et al., 2019) to legal blindness at a median age of 45 (Sandberg et al., 2007). This variability is also influenced by the definition of legal blindness used. Myopia is a very common feature, and best-corrected visual acuity decline has been reported in one study to be greater in high myopes (>6 dioptres) versus those with less refractive error (7.9% versus 3.1% loss of visual acuity per year) (Talib et al., 2019).

There are conflicting reports regarding correlation of the location of RPGR variants and disease phenotype. Some studies report worse visual function in patients with variants in exons 1–14 compared to in ORF15 (Fahim et al., 2011; Sharon et al., 2003; Yang et al., 2014b), whereas others report the opposite (Andreasson et al., 2003; Talib et al., 2019). However there may be a number of factors to consider in interpreting these data, including the phenotypic variability evident in patients in the same family with the same variant, documented variation in the

Fig. 5. The watershed zone of the RPGR ORF 15. Fundus autofluorescence (AF) images of patients from the Moorfields cohort, harboring different variants are shown next to a schematic representation of RPGR ORF15. Amino acid residues are marked according to their chemical characteristics. The mostly negatively charged part of the ORF15 contains several TTLL5 glutamylation sites, marked with green rectangles. Amino acid sequences resulting from frameshift mutations are shown next to the AF images. Patients with a rod-cone dystrophy are shown on the left and patients with cone or cone-dystrophy are shown on the right side of the figure. Generally, AF is abnormal in the central retina (with surrounding normal AF) in patients with cone or cone-rod dystrophy; in the rod-cone phenotype, the pattern is largely reversed (although the central retina can often be affected, and show abnormal AF, particularly in older individuals). The watershed zone of the ORF15 (approximately 949th-1047th residue) is associated with either rod-cone or cone/cone-rod dystrophy. Transcript NM_001034853.2 was used for variant nomenclature. F = female.

Fig. 6. Images of a 23 year old patient with RP secondary to a variant in RPGR. A,B Pseudocolour, C,D autofluorescence and E,F OCT images of right and left eyes respectively of a 23 year old male patient with rod-cone dystrophy secondary to a variant in RPGR (p.Glu809GlyfsTer25). Pseudocolour images show attenuated vessels and sparse pigmentary changes however, abnormalities are more easily detected on AF imaging with widespread areas of patchy peripheral hypoautofluorescence, and hyperfluorescence within the macula region. Visual acuity was 6/12 in each eye.
phenotype of patients with ORF15 variants (Ruddle et al., 2009) compared to those in exons 1 to 14. In addition variants in proteins associated with RPGR such as RPGRIP1, may also affect disease manifestation (Fahim et al., 2011).

4.1.2.2. Imaging findings in rod-cone dystrophy.
Fundus autofluorescence (FAF) is a useful imaging modality in assessing patients with RP. Autofluorescence is primarily derived from lipofuscin in the retinal pigment epithelium (Delori et al., 1995), following phagocytosis of photoreceptor outer segments. In areas of stress or increased metabolic activity, the AF signal may be high, whereas in areas of atrophy it is reduced (von Ruckmann et al., 1999). Patients with retinal degenerations often have a ring of increased autofluorescence in the macular region delineating the junction of healthier tissue and that with more extensive degeneration (Robson et al., 2003, 2008b). In RP, studies have shown that the area within this ring corresponds to structurally intact retinal structures on OCT and areas of preserved photopic and scotopic function assessed using pattern ERG or high spatial resolution perimetry (Hood et al., 2011). Structural changes correlate well with residual function, and the rate of constriction of the ellipsoid zone width (or area) and reduction in ONL thickness can be used as measures of assessing disease progression (Birch et al., 2013; Tee et al., 2019).

Fig. 6 depicts multimodal imaging findings in male patient aged 23 with RPGR-associated rod-cone dystrophy. Fig. 7 shows findings from a patient aged 56.

4.1.2.3. Cone and cone-rod dystrophy.
X-linked inheritance is seen only in around 1% of patients with a molecularly characterized cone-rod dystrophy (Birtel et al., 2018a), but RPGR is the causative gene in the majority of these cases (73% (Gill et al., 2019)). The predominant early symptom is deterioration in central vision (reduced acuity, colour vision, central scotomata), but may also include photophobia. Subsequently with rod system dysfunction patients will increasingly experience night blindness and peripheral visual field loss. Most patients have myopia, with 50% (Talib et al., 2019) to 72% (Thiadens et al., 2011) having a refractive error of greater than −6 dioptres.

As expected from early macula involvement, the rate of visual acuity decline is faster than in RP, with a rate of 5.5%/year in the better seeing eye, 7.9%/year in the worse seeing eye, and 9.2%/year in the presence of the ellipsoid zone.
Fig. 8. Images of a patient with cone-rod dystrophy secondary to a variant in RPGR. A,B Pseudocolour, C,D autofluorescence and E,F OCT images of right and left eyes respectively of a 54 year old male patient with cone-rod dystrophy secondary to a variant in RPGR (p.Glu1060ArgfsTer18), illustrating central atrophy on all imaging modalities. Corresponding visual acuity was 6/36 in each eye. The parafoveal and peripheral retina (denoted by arrows) appears normal on all of the imaging modalities.
of high myopia in patients over the age of 50 (Talib et al., 2019). Approximately half of patients were registered legally blind at the age of 40–50 (Talib et al., 2019; Thiidens et al., 2011).

4.1.2.4. Imaging findings in cone and cone-rod dystrophy. A ring of increased autofluorescence is also seen in many patients with cone and cone-rod dystrophy secondary to RPGR, but in contrast to those with RP, the area within the ring comprises degenerating or atrophic retina as detected via functional testing and/or assessment by OCT imaging (Birtel et al., 2018a; Robson et al., 2008a; Talib et al., 2019). As with other cone-rod dystrophies, the hyperfluorescent ring may increase in size over time as central changes worsen and the disease advances (Robson et al., 2008b).

Fig. 8 illustrates imaging findings in a patient with RPGR-associated cone-rod dystrophy.

4.1.2.5. Systemic associations. Since RPGR is expressed in a number of tissues, a small number of patients with retinal dystrophies secondary to RPGR also exhibit other signs of ciliopathies such as hearing loss, sinusitis, and chronic respiratory infections (Iannaccone et al., 2003; Zito et al., 2003).

4.1.3. Genetic testing

The majority of genes known to cause RP have good coverage using next generation sequencing techniques. However due to its highly repetitive nature, the ORF15 region of RPGR is particularly difficult to sequence accurately, including via whole genome sequencing, due to the nature of paired end short-read sequencing chemistry and alignment, and in the United Kingdom sequencing of this region needs to be carried out as an independent experiment if there is a patient whose pedigree is suggestive of X-linked RP.

4.1.4. Electrophysiology

In patients with X-linked retinitis pigmentosa (XLRP), the full-field ERG is typically severely subnormal and delayed from childhood, with young adults showing severely abnormal or undetectable dark-adapted (DA) and abnormal light-adapted (LA) responses. With reference to international standard stimuli (Robson et al., 2018), the DA 0.01 (dim flash) ERG is reduced or undetectable in keeping with loss of rod system function. The DA 10 (strong flash) ERG a-wave is subnormal or undetectable, consistent with rod photoreceptor dysfunction, with concomitant reduction in the b-wave. LA ERGs may be less severely affected initially, with delayed peak times and reductions in the LA 30Hz (flicker) and LA3 (single flash) cone ERGs. The ERG findings are consistent with a severe rod-cone or photoreceptor dystrophy (the latter term used if rod function is impaired) having lower 30Hz amplitudes on ERG compared to carriers with variant sites in exons 14 (Comander et al., 2015); and another study reports no correlation between sequence variant site and phenotype when RPGR variants were categorized by RP versus cone-rod involvement (Talib et al., 2018).

4.1.6. Current therapeutic strategies

The last 15 years have seen major advances in the field of gene therapy for inherited retinal degenerations (Kumaran et al., 2018) with landmark trials being conducted for Leber congenital amaurosis secondary to variants in RPGR (Maguire et al., 2008), and subsequent phase III trials leading to approval of the first licensed gene therapy for a retinal dystrophy (Russell et al., 2017). The vector used for gene delivery in these trials is an adeno-associated viral vector (AAV), a virus that is non-pathogenic in humans, and has a capacity of around 4.7 kb for delivery of genetic material. Several modifications can be made to this vector, such as altering the promoter or viral capsid to optimise the cell type transduced or maximize efficacy. For example, some studies have shown the AAV5 capsid having greater affinity for photoreceptors than the AAV2 vector used for RPGR gene therapy (Vandenbergh et al., 2011; Yang et al., 2002), the latter aiming to target the RPE.

A gene replacement strategy is particularly attractive in X-linked retinal dystrophies since the majority of variants result in loss of function. The RPGR ORF15 isoform has been extensively investigated for the purpose of gene therapy, since no disease causing variants have been identified in exons 16–19 (Vervoort and Wright, 2002), and also its sequence being 3459 bp can be suitably packaged in an AAV capsid.
Fig. 9. Images of female carriers of pathogenic variants in *RPGR*. A,B Pseudocolour, C,D autofluorescence and E,F OCT images of right and left eyes respectively of a 37 year old *RPGR* carrier (c.1572+1G>T) illustrating a tapetal reflex (more evident on autofluorescence imaging), with corresponding visual acuities of 6/18 in her right amblyopic eye and 6/5 in her left eye. G,H Pseudocolour, I,J autofluorescence and K,L OCT images of right and left eyes respectively of a 54 year old *RPGR* carrier (p.Glu802GlyfsTer32) illustrating patchy pigmentary and atrophic changes in all four quadrants with inter-ocular asymmetry. Visual acuity was 6/18 in each eye.
However, it has been difficult to ensure stability of the ORF15 sequence, cloning of which is intrinsically error prone due to its highly repetitive nature (Martinez-Fernandez De La Camara et al., 2018). Proof of concept studies demonstrated efficacy of gene therapy using RPGR in two naturally occurring canine models of X-linked RP (Beltran et al., 2012) and several murine models (Pawlyk et al., 2016; Wu et al., 2015). Some vectors contained variations in sequence compared to that of native RPGR without any evident toxic effects (Deng et al., 2015; Pawlyk et al., 2016). Another approach has been to use codon optimisation: since some amino acids can be encoded by multiple codons, the DNA sequence is modified to use alternative codons without changing the amino acid sequence. This results in incorporation of a wider variety of nucleotides in the ORF15 region to improve stability (Fischer et al., 2017). Expression of codon-optimised RPGR is under the GRK1 promoter.

Preliminary results are promising, with the second of these trials recently publishing outcomes from 18 patients over a 6 month follow up period (Cehajic-Kapetanovic et al., 2020). They demonstrate an improvement in visual function as measured by microperimetry in seven patients, with increased outer nuclear layer thickness in the subject with increased amino acid sequence. This results in incorporation of a wider variety of nucleotides in the ORF15 region to improve stability (Fischer et al., 2017). Expression of codon-optimised RPGR is under the GRK1 promoter.

4.2. RP2 (retinitis pigmentosa)

4.2.1. Disease mechanism

The retinitis pigmentosa 2 (RP2) gene was the second gene causing X-linked RP to be identified (Schwahn et al., 1998) and comprises five exons encoding a 350 amino acid protein. RP2 is ubiquitously expressed (Schwahn et al., 1998), found throughout the retina and in the inner and outer segments of photoreceptors (Grayson et al., 2002). RP2 is targeted to the plasma membranes of cells via an N-terminal motif (Grayson et al., 2002) and its N-terminal structure is a beta-helix that is structurally similar to cofactor C, a tubulin-specific chaperone (Kuhnel et al., 2006). The N-terminal region of RP2 also binds the small G-protein Arf-like 3 (Arl3) (Bartolini et al., 2002; Kuhnel et al., 2006), for which RP2 acts as a GTPase-activating protein (Veltel et al., 2008), and the two play a role in assembly and trafficking of membrane associated proteins at the photoreceptor cilium (Schwarz et al., 2012). This is supported by the finding of cone opsin mislocalisation and reduced rhodopsin content in the outer segments of RP2 knockout mice (Li et al., 2013) and disrupted trafficking of phosphodiesterase 6A (PDE6) and G-protein coupled receptor kinase 1 (GRK1) to the photoreceptor outer segments (Zhang et al., 2015).

4.2.2. Clinical features

Patients with RP2 variants predominantly have a phenotype of RP (Sharon et al., 2003), and this gene is responsible for around 10–20% of cases of X-linked RP (Hardcastle et al., 1999; Schwahn et al., 1998). Patients present with typical features of RP, including night blindness, visual field constriction and subsequently reduction of visual acuity. A comparison of phenotypic features between 16 patients with X-linked RP secondary to RP2 and 156 patients with RPGR-RP revealed that on average, visual acuity at all ages was lower in the RP2 group (Sharon et al., 2003). This is likely due to early macular involvement in RP2 (Fig. 10A–F), with one study demonstrating 91% of patients had features of macular involvement, with 36% having a central scotoma on Goldmann visual field testing before the age of 12 years (Jayasundera et al., 2010). Two patients in this report were also noted to have a Choroideremia-like peripheral atrophy, with testing of the choroideremia gene being negative (Jayasundera et al., 2010). A family has also been described with atypical posterior pole and macular atrophy secondary to a microdeletion in exon 3 of the RP2 gene (Dandekar et al., 2004).

Of the relatively few patients with refractive error documented in the literature, most were found to have varying degrees of myopia, with one paper reporting mean refraction of $-2.65 \pm 0.67$ diptors (Sharon et al., 2003), another of $-7.97$ diptors (Jayasundera et al., 2010). Other phenotypic features including ERG parameters were largely similar to RP patients with variants in RPGR, making it challenging to distinguish which gene is responsible for X-linked RP based on phenotype alone; although RP2 males in childhood (and carrier females in adulthood) tend to be more severe with early macular involvement.

Few RP2 carriers have been characterised, some have a tapetal reflex similar to RPGR carriers, as seen in a patient in the Moorfields cohort (Fig. 10G–J), whereas there are also reports of carriers having a similar phenotype to males with macular atrophy in one or both eyes and myopia (Jayasundera et al., 2010), personal communication Michaelides).

4.2.3. Current therapeutic strategies

Gene therapy has also been investigated as a therapeutic strategy for X-linked RP secondary to RP2. The RP2 coding sequence is 1050 base pairs in size rendering it suitable for packaging in an AAV vector. A single published study assessed delivery of RP2 to an Rp2 knockout mouse in which the phenotype is, in contrast to humans, predominantly of cone degeneration (Mookherjee et al., 2015). An AAV8 vector was delivered via subretinal injection with RP2 expression under control of a photoreceptor-specific rhodopsin kinase promoter. They demonstrated preservation of cone function over an 18 month period as evidenced by cone viability, M-opsin localisation and photopic ERG recordings (Mookherjee et al., 2015). Retinal toxicity was however demonstrated at high doses highlighting the need for careful assessment of vector dose in gene therapy studies.

A recent study developed RP2 knock-out and patient-derived induced pluripotent stem cell (iPSC) derived retinal organoids as a model of RP2 RP. Following gene therapy using an AAV5 vector, they demonstrated increased outer nuclear layer thickness and rhodopsin expression compared to controls, supporting further investigation of the gene therapy approach for RP2 RP (Lane et al., 2020).

4.3. CHM (choroideremia)

4.3.1. Disease mechanism

Variants in the CHM gene are responsible for the choriotereinal degeneration choroideremia (Cremers et al., 1990). This gene comprises 15 exons and encodes a 653 amino acid protein known as Rab escort protein 1 (REP1) (van den Hurk et al., 1997). REP1 is involved in the process of prenylation, a post translational modification that involves addition of a prenyl group to a Rab GTPase, a member of the Ras superfamily of G proteins involved in vesicle trafficking (Seabra et al., 1992, 1995). As well as facilitating prenylation, REP1 also plays a role in escorting Rab7a, have an important role in the retina (Telmachova et al., 1999).

REP1 is a ubiquitously expressed protein although the disease caused by absence of REP1 manifests in the eye alone. This is believed to be due to the presence of REP2, a protein encoded by the CHM (choroideremia-like) gene on chromosome 1, which has 90% similarity to REP1, and is also ubiquitously expressed (Cremers et al., 1994). In most cells,
Fig. 10. Images from a male patient with RP (secondary to an RP2 variant) and from the patient’s mother. A,B Pseudocolour, C,D autofluorescence and E,F OCT images of right and left eyes respectively of a 20 year old male with RP secondary to a variant in RP2 (p.Pro54AsnfsTer5) illustrating macular atrophy on OCT imaging, with corresponding visual acuities of 6/60 in the right eye and Counting Fingers in the left eye. G,H Pseudocolour and I,J autofluorescence images of the 42 year old, asymptomatic mother of the patient shown above, illustrating a tapetal reflex and patchy peripheral degeneration consistent with being a carrier of the RP2 variant.
REP2 can compensate for lack of REP1, whereas in the eye for example, Rab27a, is preferentially prenylated by REP1 (Larijani et al., 2003) and therefore REP2 may not be able to compensate for its absence.

The majority of variants detected in CHM are null mutations e.g. deletions, insertions nonsense, frameshift or splice site, with only two missense variants identified (Brown et al., 2010). Pathogenic changes may also be located in deep intronic (Caras et al., 2017; van den Hurk et al., 2003) or promoter regions (Radziwon et al., 2017) and therefore sequencing of these areas should also be considered if standard approaches do not yield a result in a patient with typical features.

4.3.2. Clinical features

Choroideremia affects approximately 1:50,000 to 1:100,000 people, with a high prevalence in Finland (Sankila et al., 1992). Males develop
Fig. 12. **Images of advanced disease in choroideremia.** A,B Pseudocolour, C,D autofluorescence and E,F OCT images of right and left eye respectively of a 62 year old male with choroideremia secondary to a p.Trp548GlyfsTer7 variant in \textit{CHM} illustrating widespread chorioretinal atrophy with an island of central retina remaining, with corresponding visual acuities of 6/13 in each eye.

Fig. 13. **Images of a patient with retinal dystrophy secondary to a dominant variant in \textit{RPE65}.** A,B Pseudocolour and C,D autofluorescence images of the right and left eye respectively of a 53 year old male with a p.Asp477Gly variant in \textit{RPE65} illustrating widespread chorioretinal atrophy and corresponding visual acuities of hand movements in the right eye and 6/60 in the left eye.
symptoms of nyctalopia in the first decade of life followed by slowly progressive visual field constriction. The majority of patients retain useful central visual function until at least the fifth to sixth decades (Coussa et al., 2012; Roberts et al., 2002), although there is variability in disease severity with reports of some younger patients having severe visual loss (Karna, 1986). Patients with choroideremia are also reported to have a generalised reduction of colour vision function evident early in disease (Jolly et al., 2015) that is not associated with level of visual acuity (Heon et al., 2016).

Fundus changes progress from pigment clumping at the level of the RPE early in disease to mid-peripheral atrophy, with peripapillary atrophy also being evident early in the disease course (Khan et al., 2016). These atrophic changes progress outwards towards the ora serrata, and also in a centripetal manner towards the fovea, often leaving a characteristic, scalloped island of intact residual tissue until end-stage disease (Hariri et al., 2019). There is also progressive atrophy of the choriocapillaris resulting in visible choroidal vessels in established disease. Fig. 11 shows retinal images from a 5 year old affected male (Fig. 11A–D) and an 18 year old male (Fig. 11E–J); Fig. 12 shows images from an older male with more advanced disease.

The clinical appearance of choroideremia is usually pathognomonic, but other conditions may mimic its fundus appearance, including retinal degeneration secondary to a dominant variant (p.Asp477Gly) in the RPE65 gene (Hull et al., 2016) (Fig. 13), variants in RHO (Audo et al., 2010), RGR (Ba-Abbad et al., 2018) PRPH2, and Oliver McFarlane syndrome (a retinal degeneration associated with trichomegaly and short stature (growth hormone deficiency) secondary to biallelic variants in the PNPLA6 gene (Hufnagel et al., 2015)). Clinical differentiation may be made by establishing mode of inheritance and non-retinal features.

Choroideremia is usually an isolated retinal disease, however syndromic associations have been noted in patients with chromosomal disruption of Xq21 or contiguous deletion syndromes. These include hearing loss, cognitive impairment, cleft lip and palate, skeletal deformities, acrokeratosis (Poloschek et al., 2008) (Mitsios et al., 2018) and a female with sensorineural deafness and primary ovarian failure (Lords-Sanchez et al., 2000).

4.3.3. Imaging findings

The area of remaining functional retina in choroideremia is clearly visible on fundus autofluorescence imaging which is therefore useful for assessing disease progression (Figs. 10 and 11). There appears to be better preservation of retina temporal to the fovea compared to nasally (Hariri et al., 2019). Analysis shows the rate of change declines with time, and that degeneration is concordant between eyes (Aylward et al., 2018; Jolly et al., 2016). However, there are also reports of asymmetry later in disease (Aleman et al., 2017).

OCT imaging demonstrates structural changes early in disease such as a mild increase in central retinal thickness in children that may represent subclinical microcystic changes (Khan et al., 2016) and changes in photoreceptor outer segments and ellipsoid zone in the presence of intact RPE (Aleman et al., 2017). There is also a transition zone between relatively preserved retina and areas of more advanced structural loss that moves centripetally with age and indicates loss of
central vision (Aleman et al., 2017). This area corresponds to a reduction in outer nuclear layer thickness (Xue et al., 2016) and may show areas of intraretinal cystic change (Khan et al., 2016), with outer retinal tubulations present in the majority of areas in eyes just outside the transition zone that are not yet fully atrophic.

OCT angiography (OCTA) has also been used to image the choroid, RPE and photoreceptor layer in patients in choroideremia, aiming to increase our understanding of which cell type is primarily affected in this condition. One study reported that RPE loss exceeded ellipsoid zone loss in nearly all eyes leading to the authors to conclude that RPE loss precedes that of photoreceptors (Jain et al., 2016).

4.3.4. Electrophysiology

Male patients show progressive rod and cone system dysfunction on full-field ERG (with earlier and greater reduction in rod-driven responses), with loss of DA10 ERG a-wave and b-wave amplitudes and delay in LA ERG peak times (Renner et al., 2006; Sieving et al., 1986). ERGs become undetectable later in life. Pattern ERG preservation is related to the area of macular sparing and may be detectable until the late stages. Multifocal ERGs have shown a pattern of dysfunction in carriers consistent with mosaicism (Vajravanant et al., 2006).

4.3.5. Carrier state

A wide spectrum of disease can be seen in female carriers (Fig. 14), ranging from mild to severe phenotypes even within the same family (Huang et al., 2012). Most carriers have good visual acuity (two thirds better than or equal to 20/40 (Coussa et al., 2012)), although this can drop to hand movements or worse in a small minority. Fundus changes can range from patchy pigment clumping to extensive atrophy; with four patterns described on autofluorescence imaging: fine, coarse, geographic and male pattern, with milder functional defects in the fine and coarse group versus the geographic and male patterns of atrophy (Edwards et al., 2015). This variability is hypothesised to be secondary to differences in the extent of skewed X inactivation.

Female carriers can show ERG responses ranging from normal, in spite of RPE pigmented changes, to mild to moderate impairment. These abnormalities typically manifest at an older age than in carriers of XLRP.

4.3.6. Current therapeutic strategies

There has been significant progress in potential treatments for choroideremia. The furthest advanced is gene therapy, where several clinical trials are currently underway, all using an AAV2 vector and a CBA promoter to drive ubiquitous expression of the CHM gene. The first phase I/II trial began in Oxford, UK in 2011 with initial results demonstrating a good safety profile (Maclaren et al., 2014). Efficacy results showed a small gain in vision in treated eyes of a median of 4.5 letters, versus a loss of 1.5 letters in untreated eyes over a 2 year period (Xue et al., 2018). Two patients had complications, one with foveal stretch during surgery and subsequent retinal thinning, and the other with inflammation that subsequently settled with an extended course of oral steroids.

Two independent trials using the same vector have shown similar efficacy (Fischer et al., 2019; Lam et al., 2019), but another showed similar rates of degeneration between treated and untreated eyes with one patient developing retinal inflammation (Dimopoulos et al., 2018). Advances in surgical technique such as the use of intra-operative OCT imaging used in all of the trials may help to minimise the risk of foveal stretch (Lam et al., 2019). The majority of patients treated had intact foveal structure at the time of treatment, and therefore improvements in acuity are believed to correspond with increased REP1 expression in RPE cells improving function of remaining foveal cones (Xue et al., 2018). A phase III trial of the vector used in the aforementioned studies is currently underway sponsored by NightstaRx Biogen (NCT03496012).

A further independent phase I/II trial sponsored by Spark therapeutics has also been undertaken (NCT02341807), with this vector being very similar to the NightstaRx vector except for the exclusion of a woodchuck hepatitis virus posttranscriptional regulatory element (WPRE) within the vector construct, which may affect levels of transcription.

In-frame nonsense variants in the CHM gene are detected in around 30% of cases (Moosajee et al., 2014), and an alternative approach is to use small molecule drugs to allow ribosomal read through of the premature stop codons that arise from these variants and prevent abnormal truncation of the protein during translation. One such drug, Ataluren or PTC124, facilitates read through of premature but not normal stop codons (Welch et al., 2007) and has been approved for use in patients with Duchenne Muscular dystrophy. Proof of concept studies have demonstrated increased rep1 production and functional improvement using in vitro prenylation assays in a zebrafish model, which is the only existing animal model of choroideremia with a nonsense mutation (Moosajee et al., 2016).

4.4. RS1 (X-linked retinoschisis)

4.4.1. Disease mechanism

The RS1 gene comprises six exons and encodes retinoschisin, a 224 amino acid protein, with variants in this gene being responsible for X-linked retinoschisis (XLSR) (Sauer et al., 1997). Retinoschisin is produced and secreted by photoreceptor and bipolar cells, and adheres to their cell surface (Molday et al., 2001). Its exact function is still unknown although it is believed to play an important role in cell adhesion and mediation of cell-to-cell interactions. This is supported by the structure of retinoschisin, which is largely comprised by a discoidin domain (aa residues 62–219), known to facilitate cell adhesion (Baumgartner et al., 1998). In addition, retinal laminar architecture becomes highly disordered in the absence of retinoschisin along with disorganisation of the photoreceptor ribbon synapse and schisis like gaps in the inner nuclear layer as seen in the rs1 knockout mouse (Weber et al., 2002).

Another proposed role for retinoschisin is the regulation of fluid balance within the photoreceptor and bipolar cell layers, evidenced by the fact that retinoschisin binds to the Na+K+ATPase pump which has a key role in controlling ion gradients and therefore osmolarity (Molday et al., 2012). A further interaction is with L-type voltage gated calcium channels such as CACNA1D (Shi et al., 2009), helping to maintain membrane localisation of these ion channels and therefore photoreceptor-bipolar cell transmission. More recently an interaction between retinoschisin and CACNA1F has been demonstrated (Shi et al., 2017), the latter being responsible for incomplete congenital stationary night blindness and this interaction may go some way to explaining the overlap of electrophysiological features between these two conditions.

4.4.2. Clinical features

This condition affects approximately 1:15,000 to 1:30,000 males (Sikkink et al., 2007), typically presenting with symptoms of reduced central vision at school age (often failing the school vision test), strabismus, or anisometropia, but a smaller number may present in infancy with strabismus, nystagmus or have bullous retinoschisis (George et al., 1995). Visual acuity can vary widely from 20/20 to 20/600 (George et al., 1996) with mean BCVA being 0.49–0.6 logMAR in recent studies (Ores et al., 2018; Yang et al., 2014a). Prognosis is often relatively good in childhood, unless retinal detachment or vitreous haemorrhage occur, which are associated with a poor prognosis.

Clinical changes at the macula include typical schitic changes, fine white dots resembling drusen-like deposits, non-specific RPE changes and macular atrophy, with the latter being seen in some older adult patients (George et al., 1996; Tsang et al., 2007). Approximately 50% of patients also have peripheral retinal changes, including a silver reflex of the internal limiting membrane (Fig. 15), and peripheral whitish or pigmentary disturbance (George et al., 1996; Vincent et al., 2013).

Peripheral retinoschisis is seen in 30–71% of patients, with retinal
detachment in 3–16% of eyes (George et al., 1996; Ores et al., 2018). A subset of patients have bullous retinoschisis who tend to present in childhood with strabismus, significantly decreased vision, nystagmus, floaters secondary to vitreous haemorrhage or an irregularly shaped pupil, and these cases may be complicated by tractional or exudative retinal detachment (Hinds et al., 2018).

### 4.4.3. Imaging

OCT imaging demonstrates macular schisis in the majority of patients (Fig. 15), with a recent study reporting foveoschisis in 78% patients and an isolated parafoveal schisis in a further 10% (Ores et al., 2018). Macular atrophy may be seen later in the disease course (Fig. 16) and the OCT appearance can be rarely normal (Vincent et al., 2013). Intraretinal cysts can be found in any layer of the retina but most...
predominantly in the inner nuclear layer, followed by the outer plexiform layer and ganglion cell layer (Ores et al., 2018). Qualitative changes are also seen in the interdigitation zone, ellipsoid zone and external limiting membrane and photoreceptor outer segments are shorter than controls in this condition.

The characteristic autofluorescence finding in patients with XLRS is a spoke-wheel pattern of high and low intensity signal due to displacement of luteal pigment, however recent studies have only identified this finding in approximately half of patients (Ores et al., 2018; Vincent et al., 2013). Other patterns include normal AF, low signal in the foveal region, an area of low signal surrounded by a ring of increased signal intensity, or irregular or regular concentric areas of high- and low-intensity FAF.

4.4.4. Electrophysiology

The characteristic finding on ERG testing in X-linked retinoschisis is reduction of the DA10 (strong flash) ERG b:a ratio, with preservation of the a-wave (an electronegative ERG occurs in the majority; b:a ratio less than 1). There is typically LA 30Hz flicker ERG delay and variable amplitude reduction of the LA ERGs (Fig. 24D). Some phenotype-genotype correlation is evident, with patients with nonsense, splice-site, or frame-shift variants in RS1 consistently demonstrating an electronegative DA10 ERG, markedly delayed LA 30Hz (flicker) ERG and an abnormal PERG P50 (Vincent et al., 2013). Missense variants in RS1 are associated with a wider range of ERG abnormalities including those with the mildest ERG phenotypes; electronegative waveforms are present in most but in a minority the b:a ratio is only mildly reduced. Pattern ERG P50 is usually subnormal in keeping with macular dysfunction, but missense changes can be associated with a normal response in a minority (Vincent et al., 2013).

4.4.5. Carrier state

In contrast to most other X-linked retinal degenerations, there appears to be no clinical disease phenotype in the carrier state for XLRS. There are a few reports of abnormalities on specific electrophysiological testing protocols in some carriers such as areas of dysfunction on multifocal ERG (Kim et al., 2007) or timing of 8Hz flicker ERG responses.
A few females however have been reported to manifest XLRs, all from consanguineous families and found on genetic testing to have homozygous variants in RSI (Ali et al., 2003; Gliem et al., 2014; Rodriguez et al., 2005; Saleheen et al., 2008).

### 4.4.6. Carbonic anhydrase inhibitors

Carbonic anhydrase inhibitors (CAIs) have been widely used in patients with XLRs (and also in macular oedema associated with RP) with the aim of reducing intraretinal cysts. The most frequently used are topical agents such as dorzolamide, however reports assessing efficacy include small patient numbers and report varying outcomes. The largest published study of 36 patients (68 eyes) using topical CAIs with the majority on dorzolamide, documented a reduction in intraretinal cysts in 66% of eyes despite only half the patients maintaining dosing at three times a day. Mean gains in visual acuity were small at 0.09 logMAR (Andreuzzi et al., 2017). A recent natural history study that included a group treated with CAIs demonstrated a small mean improvement in visual acuity (3.15 ± 7.8 letters) but with three out of 20 subjects demonstrating a >15 letter gain over 18 months follow up (Pennesi et al., 2018).

Oral acetazolamide has also been assessed in this condition. A study of 11 eyes in adults given a dose of 250 mg twice a day in patients over 60 kg (and 125 mg twice a day if under 60 kg) showed a small improvement in visual acuity (0.06 logMAR), although the reduction in central macular thickness did not reach statistical significance (Gurbaxani et al., 2014). A similar study in children reported around half the patients treated showed an improvement in structure on OCT scan but the improvement in vision was not clinically significant (Verbakel et al., 2016). Further studies are needed to determine if the reduction in intraretinal cysts seen on therapy translates to preservation of central visual function over a longer period of time.

### 4.4.7. Current therapeutic strategies

Gene therapy has been extensively investigated for treatment of XLRs. As discussed for RPRG gene therapy, there are multiple ways in which the gene therapy approach can be modified to obtain optimal efficacy including altering vector capsid, promoter and route of delivery. Proof of principle studies have been performed in murine knockout models of rs1 which have a similar phenotype to humans, with development of intraretinal cysts and a reduced ERG b/a wave ratio. These have demonstrated appropriate expression of retinoschisin and functional improvement using ERG recordings using a range of AAV vectors and promoters including AAV5-opsin promoter (Min et al., 2005), AAV2-CMV promoter (Zeng et al., 2004), AAV8-retinoschisin promoter (Park et al., 2009), and AAV2 vector with three capsid mutations with a CMV enhancer/CBA promoter (rAAV2YF-CB-hRS1) (Ye et al., 2015). Two clinical trials are currently in progress; NCT02416622 run by Applied Genetics Technology Corp (AGTC) and NCT02317887 run by the National Eye Institute (NEI). Both use an intravitreal approach (as opposed to the subretinal route used for the majority of previous trials) partly to minimise the risk of retinal detachment, given the already increased risk in these patients. The AGTC trial uses an rAAV2YF-CB-hRS1 vector whereas the NEI trial uses an AAV8 vector. The latter has a self-complementary vector design. The AAV genome is single stranded and a complementary the NEI trial uses an AAV8 vector. The latter has a self-complementary vector design. The AAV genome is single stranded and a complementary model (Zeng et al., 2004). The NEI trial uses an intravitreal approach (as opposed to the subretinal route used for the majority of previous trials) partly to minimise the risk of retinal detachment, given the already increased risk in these patients. The AGTC trial uses an rAAV2YF-CB-hRS1 vector whereas the NEI trial uses an AAV8 vector. The latter has a self-complementary vector design. The AAV genome is single stranded and a complementary sequence incorporated into the AAV vector genome which recombine on nuclear entry, bypassing this step and increasing the efficacy of gene transfer (Nakunaranjan et al., 2008).

Interim 6-month data has been released from NCT02416622 supporting safety and tolerability but without signs of clinical efficacy (AGTC, 2018). Results of a dose-escalation clinical trial involving 9 patients (3 per dosage group) from NCT02317887 was published in 2018 (Cukras et al., 2018). Visual acuity, retinal sensitivity as assessed by microperimetry and ERG amplitudes remained unchanged over the 18 month follow up period. OCT parameters were largely unchanged in most patients, however one subject in the high dose group showed sudden resolution of all intraretinal cysts at 2 weeks which persisted 2 weeks later. Four patients (one in the medium dose group and three in the high dose group) showed inflammatory responses with anterior chamber or vitreous cellular activity, all of whom responded to topical or oral steroids. All except two patients in the low dose group developed neutralising antibodies to AAV8, with high titres detected in patients in the high dose group, which has not been commonly reported in subretinal gene therapy trials. This likely reflects the difference in immune privilege between the intravitreal and subretinal space, although it is not fully established as to whether neutralising antibodies block transduction, with some reports in support of this (Kotterman et al., 2015) whereas others document good efficacy despite the presence of neutralising antibodies (Guy et al., 2017).

### 4.5. NYX (complete congenital stationary night blindness)

#### 4.5.1. Disease mechanism

The NYX gene encodes the 481 amino acid protein nyctalopin, a member of the small leucine-rich proteoglycan (SLRP) family, and was the second gene on the X-chromosome identified to cause the clinical phenotype of congenital stationary night blindness (CSNB) (Bech-Hansen et al., 2000; Pusch et al., 2000). In this case, the phenotype is of complete CSNB (cCSNB) and variants in NYX are the commonest cause of cCSNB accounting for over half of these cases (58% (Zeitz et al., 2015)). The prevalence of cCSNB in the population is rare, and exact figures are currently unknown.

Nyctalopin plays an important role in signalling between photoreceptors and ON bipolar cells. Photoreceptors release glutamate in the dark, which binds to the metabotropic glutamate receptor (GRM6) present on bipolar cell dendrites. This in turn maintains closure of the coupled transient receptor potential melanin 1 (TRPM1) ion channel, maintaining the ON bipolar cell in a hyperpolarised state. In the light, opening of the TRPM1 channel results in ON bipolar cell depolarisation (Koike et al., 2010). Nyctalopin is responsible for correct localisation of the TRPM1 channel to the synapse at dendritic tips, and studies in mouse models have demonstrated that in its absence, incorrect TRPM1 localisation leads to CSNB (Pearring et al., 2011).

#### 4.5.2. Clinical features

Clinical features of cCSNB include reduced visual acuity to a mean of 0.3–0.4 logMAR (Bijveld et al., 2013; Allen et al., 2003) and high myopia, with studies reporting average refractive error of approximately –7 dioptres (Allen et al., 2003; Bijveld et al., 2013). Nystagmus and strabismus may also be evident, and a common presentation of cCSNB is with these features in infancy before night blindness is apparent.

The vast majority of patients with cCSNB describe symptoms of night blindness, in contrast to those with incomplete CSNB. However, in general they do not class these as severe. One study that assessed night vision, including using a ‘light lab’ test to determine light intensity thresholds required for object recognition, found visual function to be poor at the lowest light levels (equivalent to starlight), but this improved significantly at slightly higher levels of illumination (moonlight), which may explain the moderate symptoms experienced (Bijveld et al., 2013). Colour vision in these patients tends to be normal or near-normal. There is no apparent correlation between genotype and phenotype in cCSNB and no documented carrier phenotype in females.

Fundus examination is normal in most cases of cCSNB, except for changes consistent with myopia. Therefore, electrophysiological testing and/or molecular screening is valuable to establish the diagnosis in these patients who often present in early childhood with nystagmus, myopia and unexplained reduced visual acuity.

#### 4.5.3. Electrophysiology

In the complete form of CSNB the rod-system mediated DA0.01 ERG is undetectable. To a DA strong flash (e.g. DA10) the ERG a-wave is typically normal or near-normal, consistent with preservation of rod
phototransduction, but the b-wave is markedly reduced, indicating a locus of dysfunction that is post-phototransduction. The cone-mediated LA 30Hz flicker and LA3 (single flash) cone ERGs show subtle but distinctive abnormalities. The LA3 ERG has a broad a-wave followed by a sharply rising b-wave lacking oscillatory potentials (OPs). These ERG waveforms are pathognomonic for generalised ON-bipolar cell dysfunction, consistent with the results of photopic ON-OFF ERG testing. The photopic ON-OFF ERGs show selective ON b-wave reduction with preservation of the OFF d-wave (Section 6). S-cone ERGs are attenuated in keeping with S-cone bipolar cell dysfunction. The conjunction of an X-linked family history with the typical ERG phenotype in a male is almost diagnostic for NYX-related disease. Other genetic causes of this phenotype are autosomal recessive, including pathogenic bi-allelic variants in TRPM1, LRIT3, GRM6 and GPR179. Identical full-field ERG abnormalities result from melanoma-associated retinopathy, owing to circulating antibodies to the TRPM1 protein (Xiong et al., 2013).

4.5.4. Current therapeutic strategies

The no b-wave (nob) mouse is a naturally occurring mutant model of X-linked CSNB and has been shown to harbor a causative variant in the nyx gene (Gregg et al., 2003). This mouse has been investigated extensively to help understand function of the nycatolpin protein, and in addition work has been done to rescue the phenotype using gene therapy. The latter is particularly difficult since bipolar cells are not readily transduced by the most commonly used AAV vectors for retinal gene therapy. One study has shown successful functional rescue of the nob mouse by expressing nycatolin in ON bipolar cells using an AAV vector containing four capsid mutations under the control of a novel Ple155 promoter (Scalabrino et al., 2015). This restored cell-to-cell signaling as measured by patch clamping ON bipolar cells, and the ERG b-wave.

4.6. CACNA1F (incomplete congenital stationary night blindness)

4.6.1. Disease mechanism

This large gene comprising 48 exons encodes a voltage-dependent Ltype calcium channel subunit alpha-1F that is expressed within the rod and cone active zones in the outer plexiform layer (Morgans, 2001). Calcium influx via this channel mediates glutamate release in the dark at the photoreceptor-bipolar cell synapse. CACNA1F was the first gene identified on the X-chromosome to cause CSNB (Bech-Hansen et al., 1998), with variants in this gene being the commonest cause of incomplete CSNB (iCSNB) (Bijveld et al., 2013; Zeitz et al., 2015). As the locus of dysfunction is within the photoreceptor pre-synaptic membrane, both ON and OFF pathways are affected (cones synapse directly with both types of bipolar cells). This is in contrast to complete CSNB, where dysfunction selectively affects ON bipolar cells.

4.6.2. Clinical features

Incomplete CSNB (iCSNB) is a non-progressive disorder that presents predominantly in males with features including reduced visual acuity, myopia, night blindness, nystagmus and strabismus. The condition is rare, and exact prevalence remains unknown. There is greater variability in phenotype compared to cCSNB, with one study of 66 male patients from families with a variant in exon 27 (p.Leu1056ProfsTer11) demonstrating that one or more characteristic features, such as night blindness, myopia or nystagmus, were absent in over 72% of patients (with all having typical ERG findings); in particular, 40% of patients did not have night vision problems (Boycott et al., 2000). A further report also documented only 54% of patients having symptoms of night blindness (Bijveld et al., 2013).

“Congenital stationary night blindness”, therefore, might be a misleading term for CACNA1F-associated disease. It has been suggested that the term “cone-rod synaptic disorder”, originally proposed for disease associated with variants in CABP4 (Littink et al., 2009), might be more appropriate, with this term then used to cover a range of genetic diseases affecting pre-synaptic processes involved in signal transmission at the photoreceptor synapse, including those associated with variants in CACNA1F, CACNA2D4, CABP4, and, more recently, RIMS2 (Mechausser et al., 2020).

With regard to visual acuity, studies reported mean values of 0.4–0.5 logMAR (Allen et al., 2003) (Bijveld et al., 2013). Refractive error ranged from a median of -4.8 dioptres (Bijveld et al., 2013) to a mean of -8 dioptres (Allen et al., 2003), however in the former study 22% of patients were found to be hyperopic. Light sensitivity was more widely reported in iCSNB, with approximately half the patients describing this symptom, and 30% describing mild and 17% severe colour defects which are less frequently reported in cCSNB. Nystagmus was detected in two thirds of patients and strabismus in 38% of patients (Bijveld et al., 2013). Fundus examination was normal except for changes consistent with myopia. Therefore, as with cCSNB, the diagnosis of iCSNB should be considered in young children presenting with reduced visual acuity, myopia and nystagmus.

Female carriers of CACNA1F variants do not usually manifest clinical signs, however carriers of some variants have been documented to have mild to moderate loss of vision (Kimchi et al., 2019) or electrophysiological changes (Michalakis et al., 2014). Very rarely females can also manifest the disease if they have bi-allelic pathogenic variants (Bech-Hansen and Pearce, 1993).

4.6.3. Electrophysiology

Full-field ERGs in incomplete CSNB (illustrated in Section 6) show a diminished, but not completely abolished, DA0.01 ERG (one of the features that distinguishes the ERG phenotype from that seen in complete CSNB). The DA strong flash (DA10) ERG has a markedly electro-negative waveform with normal or near-normal a-wave. The LA ERGs are more severely affected than in the complete form of CSNB, due to dysfunction in both the ON and OFF cone bipolar pathways. The LA 30Hz ERG is markedly subnormal and usually bifid in shape. The LA3 ERG is simplified in shape with a:b ratio close to 1, and occasionally has an electronegative appearance. Photopic ON-OFF ERGs show abnormalities of both the ON and OFF systems (Fig. 24F) in keeping with the influence of CACNA1F on glutamate release form the pre-synaptic membrane. The conjunction of these ERG findings in a male with an X-linked family history is almost diagnostic of CACNA1F-associated retinopathy. A rarer, autosomal recessive, cause of a similar but usually slightly more severe clinical and ERG phenotype occurs with pathogenic bi-allelic variants in CABP4 (Zeitz et al., 2015).

4.6.4. Other conditions associated with CACNA1F variants

Åland Island disease also known as Forsius-Eriksson syndrome, was first described in 1964 in a family living on the Åland Islands with the original report detailing myopia, astigmatism, nystagmus, dyschromatopsia, foveal hypoplasia and fundus hypopigmentation (Forsius and Eriksson, 1964). The electrophysiological phenotype is similar to cCSNB with reduced scotopic b-wave amplitude (Schubert-Bornschein type) (Hawksworth et al., 1995). The first sequencing report documented a novel 425bp deletion in CACNA1F spanning the whole of exon 30 and adjacent intronic areas as being responsible for Åland Island disease (Jalkanen et al., 2007). More recently, a novel p.Gly603Arg variant in CACNA1F has been reported to cause both iCSNB and Åland Island disease within the same family (Vincent et al., 2011) which suggests that these conditions may not be distinct, but that genetic or environmental modifiers result in phenotypic variation.

There are reports in the literature of CACNA1F being responsible for a cone-rod dystrophy with a recent summary documenting CACNA1F as being the causative variant in around a fifth of patients with X-linked cone-rod dystrophy (Gill et al., 2019). In these cases, symptoms were progressive and in some cases of childhood onset. However, there was
some overlap of features with iCSNB (Hauke et al., 2013; Huang et al., 2016; Jalkanen et al., 2006).

A single report in a Maori family describes features of a severe CSNB phenotype associated with intellectual impairment in male patients due to a variant in CACNA1F. All carrier females in this family also had changes evident on clinical examination and ERG testing (Hope et al., 2005). A single report also describes a phenotype of CSNB associated with retinal atrophy, optic disc atrophy and progressive deterioration of vision (Nakamura et al., 2003).

4.7. OPN1LW/OPN1MW (blue cone monochromatism, Bornholm eye disease)

4.7.1. Blue cone monochromatism

4.7.1.1. Disease mechanism. The OPN1LW gene is responsible for production of the long (L) wavelength or red cone opsin and the OPN1MW gene for the medium (M) wavelength or green cone opsin. OPN1LW and often multiple copies of OPN1MW are arranged in tandem array on the X-chromosome, and the proximity and high identity of these genes can lead to recombination events (Gardner et al., 2014). Only the first two genes in the array appear to be expressed (Hayashi et al., 1999). Blue cone monochromatism (BCM) arises when only rods and short (blue) wavelength-sensitive cones are functional, the latter opsin being encoded by a gene on chromosome 7. There are three main mechanisms by which this can arise: the first is a deletion on the X chromosome upstream of both OPN1LW and OPN1MW, in a locus control region required for expression of both genes (Nathans et al., 1989; Wang et al., 1992). The second is a two-step process by which there is recombination of the L and M opsin genes resulting in a single or often hybrid gene (as can be responsible for red-green “colour blindness” depending on the order of genes in the array). A subsequent missense variant (most commonly p.(Cys203Arg)) or exon deletion in this hybrid gene can lead to loss of function and therefore BCM (Gardner et al., 2009). The third mechanism is due to rare haplotypes (L/M interchange haplotypes) at polymorphic positions in exon 3 of the opsin genes that result from intermixing between L and M opsin genes. These interchange haplotypes (e.g. LIAVA, LVAVA, where L is the amino acid leucine, V is valine, A is alanine and I is Isoleucine) have been shown to result in aberrant splicing of the opsin genes and a variable degree of exon 3 skipping (Gardner et al., 2014).

4.7.1.2. Clinical features. BCM affects around 1 in 100,000 people and affected males have reduced vision and severely impaired colour vision.

Fig. 17. Imaging in a patient with blue cone monochromatism. A,B Pseudocolour, C,D autofluorescence and E,F OCT images of a 19 year old male with BCM secondary to a p.Cys203Arg variant illustrating reduced foveal thickness (Central retinal thickness on Spectralis OCT of 212 μm in the right and 222 μm in the left eye, which is reduced compared to healthy controls) and subtle ellipsoid zone changes on OCT imaging, with corresponding visual acuities of 6/24 unaided 6/9 with pinhole in each eye.
from birth, associated with nystagmus, light sensitivity and often myopia. This condition can therefore be difficult to differentiate from achromatopsia. However, a pedigree consistent with X-linked inheritance and myopia should raise a suspicion of BCM, since achromats tend to be hyperopic. Final visual acuity is in the order of 0.6–1.0 logMAR (Aboshiha et al., 2016). Fundus examination is usually normal, except for myopic changes. However, retinal pigment epithelial changes at the macula may be noted in older patients. The condition is traditionally considered to be stationary, although progression has been described in some families (Gardner et al., 2009, 2014; Michaelides et al., 2005).

4.7.1.3. Imaging. OCT imaging of patients with BCM has shown a reduced foveal thickness compared with controls (Barthelmes et al., 2006), ellipsoid zone disruption (Fig. 17) (Carroll et al., 2012) and reduced numbers of photoreceptors and shortened cone outer segments (Cideciyan et al., 2013). Adaptive optics SLO imaging has also confirmed a disrupted cone mosaic and reduction in the number of foveal cones, with a possible relationship with genotype, such that greater disruption and lack of cones is associated with the Cys203Arg missense variant compared to L/M inter-change variants (Aboshiha et al., 2016; Carroll et al., 2012). In addition, central atrophy has also been detected in older patients with BCM (Fig. 18), especially in those with an exonic deletion of a hybrid opsin gene, supporting the potentially progressive nature of this condition (Cideciyan et al., 2013; Gardner et al., 2014).

4.7.1.4. Electrophysiology. In BCM, DA ERGs range from normal to mildly subnormal. Mild reductions in the DA10 ERG a and b-waves may relate to the loss of normal DA cone system contribution, and both DA0.01 and DA10 ERGs may show mild attenuation due to high myopia. The LA 30Hz ERGs are undetectable (normally mediated by L and M cones) and LA3 ERGs are either undetectable or severely abnormal, with a simplified waveform (reflecting preservation of the S-cone system contribution to the single flash response). S-cone ERGs (elicited by blue flashes delivered on a photopic background) are relatively preserved in amplitude (Gouras and MacKay, 1990; Perlman et al., 2019). The preservation of S-cone ERG helps distinguish the disorder from common forms of achromatopsia (related to CNGB3 and CNGA3 variants), although rare GNAT2- and PDE6C- genotypes show phenotypic overlap, with a high incidence of myopia and relatively preserved S-cone ERGs (Georgiou et al., 2019; Michaelides et al., 2003).

4.7.1.5. Current therapeutic strategies. Adaptive optics SLO imaging has demonstrated that some I and M cone cell bodies still remain at the fovea albeit with rudimentary outer segments (Carroll et al., 2012; Cideciyan et al., 2013), paving the way for potential rescue of these cells using gene therapy. Proof of principle of this has been shown in an Opn1mw−/− knockout mouse model (since mice lack Opn1lw, these knockouts only have functional S cones). Subretinal gene therapy to deliver either Opn1mw, Opn1lw or a combination using an AAV5 vector and photoreceptor specific PR2.1 promoter resulted in opsin expression with appropriate localisation at photoreceptor outer segment tips. Functional restoration was also demonstrated by assessing ERG responses to middle wavelength (510 nm) photopic light flashes with improvement of b-wave amplitudes shown (Deng et al., 2018; Zhang et al., 2017). The interchange haplotype genotype may be amenable to antisense oligonucleotide therapy to correct associated aberrant splicing, as currently being employed in clinical trials for

Fig. 18. Imaging in a patient with blue cone monochromatism and macular atrophy. A,B Pseudocolour, C,D autofluorescence and E,F OCT images of the right and left eye respectively of a 53 year old male with BCM. Genetic analysis identified the presence of only one long wave opsin gene with a c.22delC variant. AF and OCT imaging illustrates central atrophy with corresponding visual acuities of 3/60 in each eye.
4.7.2. Bornholm eye disease

Bornholm eye disease is an X-linked cone dysfunction syndrome with dichromacy and myopia that was first described in a Danish family on the island of Bornholm (Haim et al., 1988). It is rare and prevalence remains unknown. The underlying mechanism appears to be of a rare combination of amino acids in exon 3 of the \textit{L} opsin gene, as specified by codons 153, 171, 174, 178, and 180. The resultant amino acids include LVAVA or LIAVA. As well as changing the amino acid sequence, the altered combination of nucleotides introduces an exon 3 splicing defect that results in reduction in levels of normal opsin (McClements et al., 2013; Patterson et al., 2018). These changes have been noted in the proximal gene of the opsin gene array on the X chromosome, and it is therefore hypothesised that the type of dichromacy manifested is dependent on expression of the second gene present. These genotypes have also been described in isolated dichromacy; it is hypothesised that a skewed L:M cone ratio results in the generalised cone dysfunction if the most plentiful cone subtype is inactivated by the aforementioned haplotype.

Clinical features include reduced visual acuity from infancy, myopia, astigmatism, and dichromacy. There is evidence on OCT and adaptive optics SLO imaging of varying degrees of reduced retinal thickness and a disrupted cone mosaic although residual cone inner segments can still be detected indicating that the cones lacking opsin still remain viable (Patterson et al., 2018). As noted above, the interchange haplotype genotype may be amenable to antisense oligonucleotide therapy to correct associated aberrant splicing, as currently in clinical trial for \textit{CEP290}-associated LCA.

4.7.3. X-linked cone dystrophy

There are few reports of X-linked cone dystrophy being caused by a p. W177R variant in exon 3 of the \textit{OPN1LW} or \textit{OPN1MW} genes, and this comprises around 8% of reported cases of X-linked cone dystrophy (Gill et al., 2019). All patients were myopic and reported onset of symptoms in the first decade with progressive loss of vision, visual acuities ranging from 6/18 to count fingers. Fundal examination ranged from mild pigmentary changes to macular atrophy in older patients (Gardner et al., 2010).

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Fig. 19. Imaging in a patient with ocular albinism. A,B Pseudocolour, C,D autofluorescence and E, F OCT images of the right and left eye respectively of a 23 year old male with ocular albinism secondary to a p.Trp292Gly variant in \textit{GPR143} illustrating pale fundi and foveal hypoplasia on OCT imaging, with corresponding visual acuities of 6/36 in each eye.
4.8. GPR143 (ocular albinism)

4.8.1. Disease mechanism

The GPR143 gene encodes a 404 amino acid transmembrane protein that is expressed on the apical aspect of the retinal pigment epithelial cells. In contrast to genes responsible for oculocutaneous albinism, the protein is not involved in melanin synthesis itself but melanocyte maturation (Cortese et al., 2005), and cells lacking GPR143 develop enlarged or misshapen melanosomes called ‘macromelanosomes’ as a consequence. The mechanisms by which GPR143 variants alter retinal development are not fully elucidated, however it is established that the binding of L-DOPA to GPR143 leads to production of pigment epithelial derived factor (PEDF) and downregulation of vascular endothelial growth factor (VEGF) (Falk et al., 2012). It is hypothesised that these alterations may affect retinal development. Furthermore, activation of GPR143 leads to release of exosomes, which are small vesicles carrying mRNA, protein and bioactive lipids that are involved in cellular communication and it is feasible that altered exosome release may also play a role in impairment of developmental processes (McKay, 2019).

4.8.2. Clinical features

Ocular albinism or Nettleship Falls albinism, affects around 1 in 150,000 males (Schnur et al., 1994). Features are present from birth and include nystagmus, reduced visual acuity, iris transillumination, a blonde fundus (Fig. 19) and foveal hypoplasia. Refractive error is common which can range from high hyperopia to high myopia (Wildsoet et al., 2000). Phenotypic variability may be seen in patients with Black or Asian ethnicity where only mild iris and fundus hypopigmentation may be evident (Fang et al., 2008; O’Donnell et al., 1978). Despite skin and hair pigmentation being normal, skin histology in affected males shows the presence of macromelanosomes (Garner and Jay, 1980).

4.8.3. Imaging

OCT imaging reveals foveal hypoplasia: loss of the foveal contour with persistence of the nerve fibre layer and ganglion cell layer across the fovea without thinning (Fig. 19), however the degree of these changes and photoreceptor thinning is variable across patients (Chong et al., 2009). Autofluorescence imaging shows absence of the normal pattern of reduction in peri-foveal autofluorescence, due to lack of macular pigment.

4.8.4. Electrophysiology

In ocular (OA) and oculocutaneous (OCA) albinism, a high proportion of fibres cross to the contralateral hemisphere at the optic chiasm. Studies in rodents have demonstrated that during development, ganglion cells that cross ipsilaterally in the optic chiasm are generated earlier than those crossing contralaterally from similar retinal regions (Drager, 1985), with the probability of axons crossing ipsilaterally declining with time. In albino rodents, there is a delay in ganglion cell production compared with pigmented animals (Ilia and Jeffery, 1996) and this may contribute higher proportions of axons crossing contralaterally in the optic chiasm (Jeffery, 1998). This contralateral predominance can be detected by assessing visual evoked potentials (VEPs), in particular by measuring the asymmetry of interhemispheric latency of

Fig. 20. Images of a carrier of ocular albinism. A,B Pseudocolour, C,D autofluorescence and E,F OCT images of the right and left eye respectively of a 15 year old female heterozygous for a p.Cys116Gly variant in the GPR143 gene illustrating patchy pigmented changes, radially orientated in the periphery (denoted by arrows), with corresponding areas of hypoautofluorescence on AF imaging. She was asymptomatic with visual acuity of 6/6 in each eye.
the pattern appearance VEP and the amplitude asymmetry of the flash VEP. The size of both of these metrics correlate with the number of clinical signs of albinism (Dorey et al., 2003). However, normal VEPs do not exclude OA or OCA. The youngest patients are more likely to show the characteristic asymmetry in flash VEPs, and so it has been suggested that these are more useful for children under 3 years, whilst both flash and pattern onset stimuli should be used for those between 3 and 6, and the pattern onset VEP becomes more discriminatory above the age of 6 (Apkarian, 1992).

4.8.5. Carrier phenotype

The majority of carrier females have good visual acuity, approximately three-quarters have iris translucency and almost all (92% (Charles et al., 1992),) have mottled or “mud-splattered” fundus pigmentation consistent with patches of random X inactivation. This appearance can be similar to that seen in choroideremia carriers and is consistent with the RPE being primarily affected in both conditions. A recent imaging study showed that around three quarters of carriers have persistence of the inner retinal layers at the fovea on OCT scan, all patients assessed had reduced foveal autofluorescence, and half had a tapetal-like reflex (Khan et al., 2018). Macromelanosomes are also detected in the majority of carriers on skin biopsy (Charles et al., 1992). Fig. 20 depicts retinal images from an ocular albinism carrier; the appearance on ultra-widefield autofluorescence is striking, and appears to be quite specific for carrier status. The foveal OCT image appears to be within normal limits.

4.8.6. Current therapeutic strategies

There are currently no effective treatments for ocular albinism, with the mainstay being correction of refractive error and management of photophobia. A clinical trial for oculocutaneous albinism was conducted using

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**Fig. 21. Retinal images from patients with X-linked Alport syndrome.** A, Infrared reflectance and optical coherence tomography (OCT) from the right macula of a 28 year old male with a pathogenic variant in COL4A5. The retina appears thinner temporally with a hyper-reflective inner limiting membrane (arrow). B, Images from a 46 year old affected male. The infrared image shows flecks which appear to correspond with more prominently hyper-reflective regions of the inner limiting membrane. The OCT shows a disturbance of the foveal contour. C, images from a 72 year old female with a pathogenic variant on one allele of COL4A5. OCT shows thinning temporally with more irregularity to the retina.
This demonstrated a small increase in visual acuity of 4 autosomal recessive inheritance). These genes encode respectively the cases, X-linked inheritance) or the

4.9. COL4A5 (Alport syndrome)

4.9.1. Disease mechanism

Alport syndrome is caused by variants in the COL4A5 gene (85% of cases, X-linked inheritance) or the COL4A3 or COL4A4 genes (15%, autosomal recessive inheritance). These genes encode respectively the α5, α3 and α4 chains of collagen type 4, and loss of any one of the alpha chains affects construction of the complete α3α4α5 triple helix (Kruegel et al., 2013). Collagen type 4 is responsible for the integrity of basement membranes, and the α3 α4 α5 structure is found in the glomerular basement membrane, cochlea, cornea (Descemet and Bowman’s membrane), lens capsule and retina (inner limiting membrane and Bruch’s membrane). Therefore, Alport’s disease manifests in these organs.

4.9.2. Clinical features

Alport syndrome affects 1 in 10,000 people; predominant clinical features consist of haematuria, renal failure, hearing loss and multiple ocular features (Savige et al., 2015). These include corneal involvement where recurrent erosions may develop due to an abnormal Bowman’s membrane, and more rarely posterior polymorphous corneal dystrophy secondary to changes in Descemet’s membrane. Anterior lenticulois is a conical, anterior protrusion of the lens, which is reported to affect up to half of men with X-linked disease and the majority of patients with autosomal recessive Alport’s syndrome (Wang et al., 2014). This can be detected via slit lamp examination or by the presence of an ‘oil droplet’ sign on retinoscopy. The main symptoms are of difficulty focussing and most patients will eventually require cataract or lens surgery.

A central fleck retinopathy may be found in patients with Alport syndrome and can vary from scattered yellowish dots or flecks to more confluent changes (Savige et al., 2015). The incidence varies between reports from 13% overall to 60% of males with X-linked disease (Ahmed et al., 2013). Many patients also have a peripheral fleck retinopathy (Shaw et al., 2007), characterised by asymmetrical patches of flecks. In both cases, visual acuity and retinal function tend to be normal or close to normal (Savige et al., 2015). Temporal retinal thinning is also common in Alport syndrome (Fig. 21), and other findings have also been documented including a macular ‘lozenge’ or dull macular reflex, foveal hypopigmentation and lamellar or full thickness or giant macular holes, the latter believed to arise from abnormal collagen fibres in Bruch’s membrane and the inner limiting membrane. Most of the retinal changes (with the exception of macular holes) appear not to significantly affect visual acuity.

Females with a COL4A5 variant, i.e. carriers of X-linked disease may show many of the clinical features detailed above; however, as with other conditions, the phenotype in females tends to be milder due to random X-inactivation.

4.9.3. Imaging

OCT imaging may demonstrate temporal retinal thinning and macular holes. A study of the dot and fleck retinopathy in a few patients detected hyperreflective changes in the inner limiting membrane (ILM) and nerve fibre layer on OCT scan that corresponded to the distribution of flecks (Savige et al., 2010). The ILM generally appears more reflective on OCT in Alport patients. Fig. 21 shows temporal thinning and a hyper-reflective ILM.

4.10. NDP (Norrie disease and familial exudative vitreoretinopathy)

4.10.1. Disease mechanism

NDP is a 28 kb gene comprising 3 exons and encodes a 133 amino acid protein called Norrin. This is a secreted cysteine-knot like growth factor that regulates retinal angiogenesis in conjunction with proteins encoded by other genes such as FZD4 and LRPS5 (Ye et al., 2009). Pathogenic variants in NDP can result in both Norrie disease and familial exudative vitreoretinopathy (FEVR) with some reports documenting the same variant resulting in different phenotypes within the same family (Kondo et al., 2007). Variants in the NDP gene have also been associated with persistent hyperplastic primary vitreous (PHPV), advanced retinopathy of prematurity and Coats disease (Sims, 1993).

4.10.2. Clinical features

4.10.2.1. Norrie disease. This rare condition is usually associated with severe visual impairment, and its prevalence remains unknown. Its features include the presence of leukocoria and/or a retrolental mass or pseudoglioma secondary to retinal dysplasia being detected in infancy, with subsequent development of partial or complete retinal detachment. Cataract, posterior and anterior synechiae with secondary angle closure may also develop and ultimately, corneal opacification and phthisis may occur (Sims, 1993). The majority of affected males also develop progressive sensorineural hearing loss, up to half of patients have developmental delay or intellectual impairment, and peripheral venous insufficiency has also been rarely associated (Michaelides et al., 2004).

Rarely, female carriers of NDP variants that cause severe disease in affected males, have been reported to have retinal findings (Lin et al., 2016; Yamada et al., 2001).

4.10.2.2. X-linked familial exudative vitreoretinopathy. Familial exudative vitreoretinopathy (FEVR) is a disorder of retinal angiogenesis resulting in abnormal or incomplete vascularisation of peripheral retina. A number of associated genes have been identified including the NDP gene and autosomal genes such as FZD4, LRPS5, TSPAN12 and KIF11.

The clinical phenotype can be highly variable from mild vascular changes in the peripheral retina only to retinal neovascularisation, traction, a dragged macula (Fig. 22A), retinal folds and detachment. There may be inter-ocular asymmetry. The clinical signs are similar to those found in retinopathy of prematurity; however, those with FEVR are not prematurity. Female carriers of NDP variants may also show a disease phenotype (Shastry et al., 1999).

4.11. Other X-linked retinal degenerations

4.11.1. IKBKG (incontinentia pigmenti, IP)

IP or Bloch-Sulzberger syndrome is an X-linked dominant condition that has an incidence of 0.7 per 100,000 (Fusco et al., 2014) and is usually lethal in males. IP arises due to a variant in the IKBKG or NEMO gene, which is usually a deletion encompassing exons 4 to 10 (Smahi et al., 2000), and three-quarters of cases are sporadic (Fusco et al., 2014). The main diagnostic feature comprises skin changes which progress from a neonatal vesiculobullous eruption (stage I), to a verrucous stage (Stage II), hyperpigmentation (Stage III), and finally hypopigmented areas (Stage IV) that follow Blaschko lines (Fusco et al., 2014; Landy and Donnai, 1993). Central nervous system involvement including seizures and developmental delay occur in around 30–40% of patients. Other systemic features include teeth abnormalities such as delayed primary dentition or cone shaped teeth, alopecia and nail dystrophy (Fusco et al., 2014; Meuwissen and Mancini, 2012).

Ocular features have been described in 35–77% of patients with varying severity (Carney, 1976; Holmstrom and Thoren, 2000). If significant loss of vision occurs, this is often unilateral or shows inter-ocular asymmetry (Fig. 22C and D) (Holmstrom and Thoren, 2000;
O’Doherty et al., 2011). Anterior segment signs can range from corneal opacities and cataract to a shallow anterior chamber, iris hypoplasia, corneal decompensation and phthisis. Posterior segment changes range from non-specific RPE changes to a vascularized or non-vascularized ridge temporally, sometimes with retinal or vascular traction, fibrosis or areas of avascular retina peripheral to the ridge (Fig. 22E and F), and dragging of central vessels. In severe cases, retinal detachment or a retrolental mass may occur. Optic atrophy has also been reported in a few patients (Holmstrom and Thoren, 2000; Meuwissen and Mancini, 2012).

4.11.2. Aicardi syndrome

Aicardi syndrome is a rare, X-linked dominant neurodevelopmental disorder with a reported incidence of approximately 1 in 100,000 births (Kroner et al., 2008). The vast majority of patients are females and the causative gene is as yet unknown, with all cases being de novo and no definitive evidence of multiple cases within a family except for identical twins (Pons and Garcia, 2008). The classic clinical triad is of corpus callosum agenesis, chorioretinal lacunae and infantile spasms, although more recent diagnostic criteria include a broad range of neurological and systemic abnormalities as supporting features (Aicardi, 2005). Chorioretinal lacunae (Fig. 22B) are usually located in the peripapillary area, are yellow or white in appearance, round in shape, of variable size, and do not enlarge over time. The majority of patients also have optic nerve abnormalities such as colobomata (Wong and Sutton, 2018).

4.11.3. PRPS1 (photoreceptor dystrophy)

The PRPS1 gene (phosphoribosyl pyrophosphate synthetase 1) has been shown to cause an X-linked retinal degeneration with interocular asymmetry. Missense variants in this gene usually cause Arts syndrome,
Fig. 23. Expression by retinal cell type of X-linked retinopathy genes listed in Table 1. Data are from the single-cell transcriptome study of Lukowski et al. (2019), plotted from the expression levels given in their supplementary material ("Dataset EV2") following canonical correlation analysis. Bars representing the same cell type have been grouped and given the same colour.
Charcot–Marie–Tooth, and non-syndromic sensorineural deafness. Symptoms include night blindness, light sensitivity and reduction in vision, the latter being highly variable with a few patients reported with severe visual loss and others with relatively good visual acuities (Almoguera et al., 2014; Fiorentino et al., 2018). Patients may also have hearing loss or systemic abnormalities. Fundus features reported include patchy mid-peripheral atrophy with interocular asymmetry, macular atrophy in some patients and optic disc atrophy in the majority of patients. These reports unusually showed disease manifestation in females only, with no affected males, suggesting skewed X-inactivation in females, which was confirmed in one patient only (Almoguera et al., 2014). It may be that the hemizygous state in males results in a lethal outcome. It is possible that they have a more recessively inherited condition. It is also possible that the phenotypic expression of the disease involves more than one gene. Data from the available pedigrees indicate that the condition can arise in females as well as males and most affected cases are familial.

Electrophysiological assessment has revealed a range of ERG phenotypes including generalised retinal dysfunction with a similar degree of cone and rod photoreceptor involvement, worsening rod function, cone-rod dystrophy and cone dystrophy. There may be significant interocular asymmetry and normal ERGs have also been reported. Pattern ERG testing of macular function is in keeping with severe macular involvement (Fiorentino et al., 2018).

4.11.4. OFD1 (retinitis pigmentosa) A single report has identified OFD1 to be a cause of a severe form of X-linked RP. Disease-causing variants in this gene are usually associated with the ciliopathy known as orofaciodigital syndrome-1; however, a variant within intron 9 that creates a cryptic exon and subsequent frameshift, has been reported to cause a phenotype of RP (Webb et al., 2012). A variant in OFD1 has also been associated with X-linked Joubert syndrome, with one of two families characterised showing retinal degeneration amongst other features of this condition (Coene et al., 2009).

5. Expression levels of relevant genes in human retina by cell type Lukowski et al. recently reported results from single-cell RNA sequencing of over 20,000 retinal cells from three human donors, constructing a transcriptome atlas (Lukowski et al., 2019). They found 18 transcriptionally distinct cell populations that encompassed all known retinal neuronal types. Their supplementary material gives an exhaustive list of genes and their relative expression levels in the different cell types. We explored this important reference dataset for data relating to expression of known genes associated with X-linked retinopathies.

Fig. 23 shows the results of this investigation, plotting expression levels for all of the genes in Table 1 for each of 13 clusters (obtained by Lukowski et al. following canonical correlation analysis to correct for batch effects). Bars representing clusters from the same neuronal cell type have been given the same colour. As these are from the neural retina, RPE cells are not included (and some conditions are primarily a consequence of RPE dysfunction). Also, expression in a neuronal cell type does not necessarily indicate that dysfunction of that particular cell is responsible for disease: for example, in choroideremia, the CHM gene encoding the REP1 protein is expressed ubiquitously (Section 4.4.1), but the RPE appears to be the primary site of disease. Nevertheless, potentially useful insights can be gleaned from Fig. 23. CACNA1F is expressed in bipolar cells as well as photoreceptors. Whilst the ERG abnormalities likely arise from impaired synaptic transmission from photoreceptors to bipolar cells (since onward synaptic transmission from bipolar cells does not play a major role in generating flash ERG a-waves and b-waves, though would affect the later photopic negative response), impairment of transmission at the second order (bipolar cell) synapse might contribute further to visual impairment. NYX, on the other hand, is expressed solely in bipolar cells, as expected; here the mechanism of disease is impairment of the usual ON bipolar cell depolarisation secondary to photoreceptor hyperpolarisation in response to light.

A number of the genes appear to be highly expressed in Müller cells, consistent with an important role of this cell type in maintenance of retinal structure and function, despite not being directly involved in the visual signalling pathway. The main retinal cell-type expressing COL4A5 is the Müller cell. Endplates of these cells form the inner limiting membrane, and so this pattern of expression might explain the abnormal ILM noted in Alport patients (Sections 4.9.2 and 4.9.3). Müller cells are also intimately involved in retinal vasculature, and the relatively high expression of NDP in these cells might be of relevance to both Norrie disease and FEVR.

RPGR and RS1 have relatively high levels of expression in photoreceptors, as expected, although interestingly RS1 is expressed more in cones than rods; RPGR, on the other hand, is expressed more highly in some rod populations than in cones. RP2 is also expressed in photoreceptors, the site of primary pathology; a high level of expression is seen in microglia, though this might not be relevant to the disease process.

Understanding sites of expression of these genes may inform studies investigating potential therapeutic targets or reinforce the rationale behind the cell type that is targeted in approaches such as gene therapy, as well as yielding further mechanistic insights. It will also be interesting to investigate whether these patterns of expression are consistent with findings from other current or future single-cell transcriptome studies.

6. Further insights from in vivo electrophysiology

6.1. Characterisation of disease by electrophysiology

As detailed in Section 4, electrophysiology is useful in characterising many of the X-linked retinopathies. In some conditions, electoretinographic findings, in combination with a history of X-linked disease, can be almost diagnostic for a particular gene (CACNA1F and NYX). Fig. 24 illustrates typical ERG findings in a number of the conditions discussed in Section 4. As well as conventional international standard full-field dark-adapted and light-adapted stimuli (McCulloch et al., 2015; Robinson et al., 2018), responses are shown for pattern stimuli (Bach et al., 2013) and extended protocols including dark-adapted red flash (Thompson et al., 2018), light-adapted S-cone (Perlman et al., 2019) and ON-OFF ERGs (Sustar et al., 2018). ERGs should be interpreted in light of appropriate reference ranges, given known association with age in healthy individuals demonstrated by studies in our and other laboratories (Bhatti et al., 2017; Neveu et al., 2011).

6.2. Shaping of the dark-adapted a-wave trough by photoreceptor currents: insights from NYX-associated disease

The normal dark-adapted ERG response to a bright flash response comes mainly from the rod system with a relatively small contribution from the cone system. The a-wave arises largely from the photoreceptors: light leads to the reduction in the dark current entering through cyclic nucleotide gated channels in the photoreceptor outer segment, which results in a hyperpolarisation of the cell (and the negative deflection representing the a-wave of the flash ERG). The b-wave arises largely from ON bipolar cells (rods synapse with ON bipolar cells, whilst cones synapse with both ON and OFF bipolar cells, which depolarise and hyperpolarise respectively in response to light). The negative peak (or trough) of the a-wave elicited by a bright flash was thought for some time to be shaped by the intrusion of the ON bipolar cell response, whilst the photoreceptor response was assumed to continue to increase. Mathematical models developed to describe changes in photoreceptor outer segment current in response to light (Lamb and Pugh, 1992) were applied to ERG a-waves with this assumption (Breton et al., 1994; Hood and Birch, 1993; Mahroo et al., 2012).

More recently it has been shown that this assumption is likely to be
(caption on next page)
following the negative peak (Robson and Frishman, 2014). The bright fit to the whole of the a-wave trough, including the initial recovery is coming from currents within the photoreceptors; a similar waveform is seen in complete CSNB (often associated with mild rod and cone dysfunction and with a normal visual acuity (B); RPGR-related cone-rod dystrophy (C); X-linked retinoschisis (D); complete CSNB (E); incomplete CSNB (F); blue-cone monochromacy (H) and normal examples for comparison (G includes normal example of an On-Off ERG; an example of a normal DA red flash ERG (I) is shown for comparison with case H, lacking the cone-mediated x-wave). All recordings showed a high degree of inter-ocular symmetry and are shown for one eye only.

As discussed in Section 4, females carrying a pathogenic variant in XLRP genes can show a variable phenotype ranging from a tapetal reflex to RP, which might show asymmetry on clinical examination. ERG parameters can frequently be abnormal. Fig. 25 plots light-adapted 30 Hz peak times and dark-adapted strong flash a-wave amplitudes for 39 obligate XLRP carriers. These parameters are frequently abnormal, with some individuals showing a degree of asymmetry. ERGs can be helpful in these patients to establish carrier status when clinical signs might be subtle. Also, asymmetry in retinal dysfunction where present, might prompt suspicion for X-linked carrier status, as opposed to autosomally inherited RP in an affected female.

### Table 2

| Gene       | Carrier phenotype                                                                 | Figure |
|------------|-----------------------------------------------------------------------------------|--------|
| RPGR       | Variable fundus appearance, ranging from normal to a tapetal reflex, focal or patchy pigmenry changes/atrophy or more extensive intraepithelial pigment changes/atrophy. Disease often asymmetrical between eyes. | 9      |
| RP2        | Variable phenotype, ranging from tapetal reflex to male pattern pigmenry changes with macular atrophy. | 10 G-J |
| CHM        | Variable phenotype from patchy pigment clumping to more extensive atrophy. Four patterns described on autofluorescence imaging: fine, coarse, geographic and male pattern. | 14     |
| RS1        | No carrier phenotype on fundus imaging.                                           |        |
| NYX        | No carrier phenotype on fundus imaging.                                           |        |
| CANCA1F    | No carrier phenotype on fundus imaging.                                           |        |
| OPN1LW/OPN1MW | No carrier phenotype on fundus imaging.                                         |        |
| GPR143     | “Mud-splattered” fundus pigmenry changes. May have iris transillumination.        | 20     |
| NDP        | Carriers of variants causing severe disease in males rarely documented to manifest fundus changes including abnormal retinal vasculature and retinal detachment. |        |
| COL4A5     | May show clinical features associated with disease including renal impairment, hearing loss, flesy retinopathy, retinal thinning, abnormal inner limiting membrane. | 21C    |

Fig. 24D, second panel) is consistent with this: there is recovery towards baseline following the a-wave trough (though the waveform remains electronegative). This recovery thus cannot be attributed to signals from ON bipolar cells. In our studies, even when the dark-adapted cone system response is removed by subtraction (using similar methods to (Mahroo et al., 2012) and (Brigell et al., 2020)), this recovery is still seen, indicating a likely origin within the rod photoreceptors.

### 6.3. Electroretinography in XLRP carriers

Fig. 24. Examples of full-field ERGs and pattern ERGs (PERG), recorded from patients with X-linked retinal disorders (A-F, H) and from a representative unaffected control subject for comparison (G & I). ISCEV DA ERGs are shown for white flashes of 0.01, and 10.0 cd s.m⁻² (30Hz and 2Hz). Additional ERGs performed according to ISCEV extended protocols are also shown (On-Off ERG, S-cone ERG, DA red flash ERG). PERGs were recorded to alternating checkerboard stimulus and P50 used as a measure of macular function. All traces from patients are superimposed to demonstrate reproducibility. Broken lines replace blink artefacts that occur soon after b-waves in DA10 ERGs. Examples are shown of the following; 15 year old male with RPGR-related rod-cone dystrophy with severe PERG P50 reduction indicating severe macular involvement (A); 44-year old heterozygous mother of case A, showing evidence of milder rod and cone dysfunction and with a normal visual acuity (B); RPGR-related cone-rod dystrophy (C); X-linked retinoschisis (D); complete CSNB (E); incomplete CSNB (F); blue-cone monochromacy (H) and normal examples for comparison (G includes normal example of an On-Off ERG; an example of a normal DA red flash ERG (I) is shown for comparison with case H, lacking the cone-mediated x-wave). All recordings showed a high degree of inter-ocular symmetry and are shown for one eye only.
7. Future directions and conclusions

7.1. Range of diseases, mechanisms and clinical features; key messages

We have reviewed a diverse range of conditions giving rise to abnormalities in retinal structure or function secondary to pathogenic variants in X-linked genes, concentrating on non-syndromic conditions. These include largely stationary disorders (such as those associated with \textit{NYX}, \textit{CACNA1F} and \textit{GPR143}) and progressive degenerations (\textit{RPGR}, \textit{RP1}, \textit{CHM}). Variation is also seen in the primary locus of dysfunction in the X-linked retinopathies, with some first affecting the RPE (such as choroideremia), others affecting photoreceptors (\textit{RPGR}, \textit{RP2}; L and M cones in \textit{OPN1LW/OPN1MW}-associated disease) and others transmission at the first synapse of vision (between photoreceptors and bipolar cells; \textit{CACNA1F}, \textit{RS1}, \textit{NYX}). Variants in other genes affect vasculature (\textit{NDF}) or specific aspects of retinal structure (\textit{COL4A5}). Analysis of our large molecularly characterized cohort at Moorfields Eye Hospital revealed that 14% of families overall (and 21% of families in the paediatric cohort) had X-linked disease, with \textit{RPGR}, \textit{RS1}, \textit{CHM}, \textit{CACNA1F} and \textit{RP2} accounting for the majority of these patients. Given that most X-linked \textit{RP} and X-linked cone-rod dystrophy is attributable to variants in \textit{RPGR}, specific consideration should be given to sequencing the highly repetitive ORF15 region as variants can be easily missed, even in whole genome sequencing. We also found the number of families affected was significantly correlated with gene transcript length, perhaps reflecting that there is greater potential numerically for loss of function mutations to occur in longer genes (and much of X-linked disease is secondary to loss of function).

Variable X-inactivation in females can give rise to diverse carrier phenotypes. We identified a number of females with visual loss secondary to pathogenic variants in \textit{RPGR}, \textit{RP1} and \textit{CHM} and this can lead to possible misclassification of inheritance as autosomal dominant. Examination of female family members for carrier signs can also aid narrowing of the differential diagnosis. As mentioned in Section 2, we recently showed that a family in which disease had been attributed to a dominantly inherited variant in \textit{GUCA1A} was in fact more likely to be associated with a disease-causing variant in \textit{RPGR ORF15} (Mahroo et al., 2019). The reanalysis was prompted by findings of carrier features in a female relative of the proband presumed to have autosomal disease. The reported variant in \textit{GUCA1A} is likely to be benign; which has implications for other patients with this variant who would otherwise be classified as having \textit{GUCA1A}-associated dominant disease. We therefore recommend that X-linked disease always be considered unless there is evidence of male-to-male transmission.

Features of carrier status can also be seen in the absence of symptoms such as in the case of ocular albinism, where a distinct asymptomatic carrier phenotype is routinely observed. Table 2 summarises signs associated with carrier status. Interestingly, no carrier signs are seen on clinical examination or retinal imaging in patients with X-linked retinoschisis. The \textit{RS1} protein product is secreted extracellularly, and it is tempting to speculate that in females carrying a pathogenic variant, production of protein from the healthy X chromosome (active in roughly 20% of cells) perhaps reflecting that there is greater potential numerically for loss of function from the healthy X chromosome.

Fig. 26. Possible algorithm for investigating male patients with non-syndromic retinopathies and an X-linked family history, to guide genetic investigations or their interpretation. A clinical history (including the type, age, and order of onset of symptoms, as well as presence of progression) is important. Retinal imaging (spectral domain optical coherence tomography and fundus autofluorescence imaging) is useful in all patients. Note that foveal hypoplasia and atrophy are not necessarily correlated with gene transcript length.
should also be considered.

ways clear, so a number of autosomal (and even mitochondrial) genes respectively, as testing of large gene panels by massively parallel sequencing understanding of which cellular current flows give rise to the healthy ERG, trophysiological findings in these conditions can also improve our un discernible.

-half of cells) is sufficient to support normal or near-normal function, such that clinically and structurally no carrier signs are readily discernible.

Electrophysiological testing is useful in characterizing disease and establishing the diagnosis, with some conditions having a near pathognomonic electrophysiological signature, such as those primarily affecting a specific class of photoreceptor or affecting transmission at the photoreceptor to bipolar cell synapse. This is particularly important in subtypes of the latter, such as CACNA1F and NYX associated disease, where imaging might be largely normal. Visual evoked potentials are needed to elicit signs of chiasmal misrouting in ocular albinism. Electrophysiological findings in these conditions can also improve our understanding of which cellular current flows give rise to the healthy ERG, with the example discussed above of recordings in NYX consistent with the shaping of the strong flash a-wave trough by currents within the photoreceptors rather than the ON bipolar cells. This in turn will aid further refinement of the ERG as a tool in delineating mechanisms in diseases in which the primary cellular sites of impairment are not understood.

7.2. Narrowing the differential diagnosis to guide genetic testing or its interpretation

In light of the phenotypic features, retinal imaging (OCT and FAF) and in some cases, electrophysiological investigations discussed above, an algorithm can be constructed to guide clinicians presented with a male with a non-syndromic retinopathy and a pedigree suggestive of X-linked inheritance (Fig. 26). This can be useful in deciding which genes to screen (and to consider ORF15 where RPGR is implicated). Alternatively, as testing of large gene panels by massively parallel sequencing methods is increasingly available together with whole genome sequencing, the algorithm can guide the interpretation of the inevitably large number of rare variants found, as to which genes are consistent with the phenotype. In clinical practice, an X-linked pedigree is not always clear, so a number of autosomal (and even mitochondrial) genes should also be considered.

7.3. Future directions

A number of novel therapies for retinal dystrophies are currently in clinical trials, and these are likely to increase in coming years. Approaches can be broadly categorized as those aiming to slow the rate of degeneration and minimise loss of visual function, and those aiming to restore visual function in end-stage disease, although there is a degree of overlap. Several approaches have been covered in detail in recent reviews (Dias et al., 2018; Gagliardi et al., 2019; Garafalo et al., 2019; Jin et al., 2019; Singh et al., 2020).

As discussed above, gene replacement therapy is the most advanced therapeutic approach for variants that cause a loss of function, and there is already a licensed gene-replacement treatment available for RPE65-associated autosomal recessive disease. Table 3 summarises current gene therapy trials for X-linked retinopathies. Other methods are in different stages of development, and have not yet been widely investigated for X-linked retinopathies. These include anti-sense oligonucleotides, which are small molecules that alter RNA expression, and can be used for example by redirecting splicing in disease causing variants that induce aberrant splicing. Clinical trials are underway using this approach for a variant in the CEP290 (ClinicalTrials.gov NCT 03913143) (Cideciyan et al., 2019) and USH2A genes (NCT 03780257). The rapidly progressing field of gene editing, using techniques such as clustered regularly interspaced short palindromic repeats (CRISPR) with the endonuclease cas-9 (Bakondi et al., 2016; Giannelli et al., 2019; Sgouros et al., 2020) or RNA editing (Fry et al., 2020), is also promising. This may be particularly useful for pathogenic variants that cause a gain of function, as these have been more difficult to address using alternative approaches (Bakondi et al., 2016; Giannelli et al., 2018; Tsai et al., 2019). The first CRISPR clinical trial for inherited retinal disease is now recruiting, which excises a splice variant (c.2991+1655A>G) in the CEP290 gene (ClinicalTrials.gov Identifier: NCT03872479). Other approaches being investigated include local or systemic medications aimed at improving cell survival, such as the RPE65 enzyme inhibitor Emixustat, that aims to slow the build up of toxic deposits in Stargardt disease (NCT03772665).

Current approaches aiming to restore visual function in end-stage disease are largely independent of the underlying genetic cause. These include optogenetics, i.e. introducing a photosensitive protein to remaining inner retinal cells to make them light sensitive in the absence of photoreceptors. Efficacy of this approach has been demonstrated in animal models using a number of photosensitive proteins including channelrhodopsin, halorhodopsin, melanopsin, rhodopsin and cone opsin (Bi et al., 2006; Busskamp et al., 2010; Cehajic-Kapetanovic et al., 2015; De Silva et al., 2017; Lin et al., 2008), and two clinical trials investigating types of channelrhodopsin are underway (NCT03326336, NCT03252847).

Table 3 Table of gene therapy clinical trials for X-linked retinopathies that have been conducted or are currently recruiting patients.

| Gene  | ClinicalTrials.gov Identifier | Vector | Delivery | Phase | Sponsor |
|-------|-------------------------------|--------|----------|-------|---------|
| RPGR  | NCT03252847                   | AAV2/S human rhodopsin kinase promoter | Subretinal | Phase 1/2 | MeiraGTx |
| RPGR  | NCT03116113                   | AAV8   | Subretinal | Phase 1/2 | NightstaRx Ltd/Biogen Inc. |
| RPGR  | NCT03316560                   | AAV2yY | Subretinal | Phase 1/2 | Applied Genetic Technologies Corp (AGTC) |
| CHM   | NCT01461213                   | AAV2-REP1 GRK1 promoter | Subretinal | Phase 1/2 | University of Oxford |
| CHM   | NCT02553135                   | AAV2-REP1 | Subretinal | Phase 2 | University of Miami |
| CHM   | NCT02077361                   | AAV2-REP1 | Subretinal | Phase 1/2 | University of Alberta |
| CHM   | NCT02671593                   | AAV2-REP1 | Subretinal | Phase 2 | University Hospital Tuebingen |
| CHM   | NCT02407678                   | AAV2-REP1 | Subretinal | Phase 2 | University of Oxford |
| CHM   | NCT03496012                   | AAV2-REP1 | Subretinal | Phase 3 | NightstaRx Ltd/Biogen Inc. |
| CHM   | NCT03507686                   | AAV2-REP1 | Subretinal | Phase 2 | NightstaRx Ltd/Biogen Inc. |
| CHM   | NCT02341807                   | AAV2-bCHM | Subretinal | Phase 1/2 | Spark Therapeutics |
| RS1   | NCT02317887                   | AAV2 human rhodopsin kinase promoter with IRBP enhancer element | Intravitreal | Phase 1/2 | National Eye Institute (NEI) |
| RS1   | NCT02416622                   | AAV2yY CMV enhancer/CBA promoter | Intravitreal | Phase 1/2 | Applied Genetic Technologies Corp (AGTC) |
NCT02556736). Stem cell-mediated replacement has also been extensively investigated with the aim of reconstructing the cells that have degenerated (Gagliardi et al., 2019; Singh et al., 2020). In addition, several clinical trials have been undertaken using both subretinal and epiretinal electronic implants to directly stimulate remaining retinal cells (Ahuja and Behrend, 2013; Luo and da Cruz, 2016; Yue et al., 2016), demonstrating proof of principle that stimulating residual retinal cells can restore visual function.

Many questions remain unanswered. The variability of disease phenotypes in males with the same genotype (for example the same RPGR variant giving rise to a disease causing primary rod death in some family members and primary cone loss in others) requires further investigation. Identification of modifiers that give rise to different levels of severity could shed light on potential protective mechanisms to slow disease. Processes governing X-inactivation are also not fully understood, and the extent to which this can explain phenotypic variability in females and how easily this can be predicted (or influenced) warrants exploration.

The role of different cell types in disease can be investigated further. CACNA1F for example is expressed not only in photoreceptors but in bipolar cells (Fig. 23), and it is possible that the latter site of expression contributes additionally to visual impairment. Understanding how different components of the retina’s electrical response are shaped (as yielded from a study of ERGs in complete CSNB for example) will also aid understanding of conditions in which these particular features are selectively altered and how different cellular current flows affect visual signal transmission. Refinement of ERG protocols based on these insights will allow more precise functional assessment of aspects of retinal neuronal function, yielding novel quantitative ways of assessing efficacy of experimental therapies.

Myopia, to different degrees, is a significant feature of several X-linked conditions, to the extent that its presence often raises the possibility of some X-linked inherited retinal diseases, (including disease associated with RPGR, RP2, NYX, CACNA1F, OPN1LW/OPN1MW), but not others (RS1, COLA5, for example). Interestingly, heterozygous variants in ARR3 have been associated with early-onset high myopia limited to females. ARR3 resides on the X-chromosome and encodes the form of arrestin expressed in cone photoreceptors (playing a role in termination of the photoreceptor). The males in those families, who had hemizygous variants, were not highly myopic (Liu et al., 2020; Xiao et al., 2016). It is tempting to speculate that the presence of cone populations with different response kinetics might be driving development of myopia in the affected females (who, due to X-inactivation, would be assumed to have both affected and unaffected cones), whilst the males have a cone population with uniform kinetics.

A study of which monogenic inherited retinal diseases give rise to myopia (and to what extent) might shed light on the processes underlying emmetropisation and those driving the development of refractive error in a particular direction (Hendriks et al., 2017). The dramatically increasing prevalence of myopia worldwide (approximately 30% in Europe (Williams et al., 2015) and over 70% of young adults in some East Asian countries (Dolgin, 2015)) has been identified as a public health concern, particularly in view of the increased risk of sight-threatening complications. Identifying the mechanisms driving myopia could inform the development of preventive interventions. Thus, further investigation into mechanisms and phenotypes in inherited retinal disease, including the X-linked conditions which comprise a significant proportion, may yield insight not just into these diseases, but into retinal physiology and pathophysiology more generally, including in relation to the common ocular disorder, refractive error.

CRediT authorship contribution statement

Samantha R. De Silva: Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing - original draft, Writing - review & editing. Gavin Arno: Conceptualization, Data curation, Formal analysis, Funding acquisition, Resources, Software, Supervision, Validation, Visualization, Writing - review & editing. Anthony G. Robson: Data curation, Formal analysis, Visualization, Writing - review & editing. Ana Fakin: Data curation, Validation, Visualization, Writing - review & editing. Nikolaos Pontikos: Data curation, Formal analysis, Funding acquisition, Software, Validation, Writing - review & editing. Moin D. Mohamed: Resources, Supervision, Writing - review & editing. Alan C. Bird: Conceptualization, Supervision, Writing - review & editing. Andrew R. Webster: Conceptualization, Funding acquisition, Investigation, Methodology, Resources, Supervision, Writing - review & editing. Michel Michaelides: Conceptualization, Funding acquisition, Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing - review & editing. Omar A. Mahroo: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing.

Declarations of competing interest

none

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