Cold Spring Harbor Perspectives in Medicine
www.perspectivesinmedicine.org

Choroideremia: Toward Regulatory Approval of Retinal Gene Therapy

Imran H. Yusuf[1,2] and Robert E. MacLaren[1,2]

[1]Nuffield Laboratory of Ophthalmology, Department of Clinical Neurosciences, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DU, United Kingdom

[2]Oxford Eye Hospital, John Radcliffe Hospital, Oxford University Hospitals NHS Foundation Trust, Oxford OX3 9DU, United Kingdom

Correspondence: enquiries@ndcn.ox.ac.uk

Choroideremia is an X-linked inherited retinal degeneration characterized by primary centripetal degeneration of the retinal pigment epithelium (RPE), with secondary degeneration of the choroid and retina. Affected individuals experience reduced night vision in early adulthood with blindness in late middle age. The underlying *CHM* gene encodes REP1, a protein involved in the prenylation of Rab GTPases essential for intracellular vesicle trafficking. Adeno-associated viral gene therapy has demonstrated some benefit in clinical trials for choroideremia. However, challenges remain in gaining regulatory approval. Choroideremia is slowly progressive, which presents difficulties in demonstrating benefit over short pivotal clinical trials that usually run for 1–2 years. Improvements in visual acuity are particularly challenging due to the initial negative effects of surgical detachment of the fovea. Despite these challenges, great progress toward a treatment has been made since choroideremia was first described in 1872.

Choroideremia is rare X-linked recessive inherited retinal degeneration resulting from mutations in the *CHM* gene on the long arm of the X chromosome (Xq21.2) (OMIM: 300390) (Cremers et al. 1990a,b,c). The disease affects approximately one in 50,000 males, although may be more common in Finland (~1 in 40,000) (Sankila et al. 1992). *CHM* encodes REP1 (Rab escort-binding protein 1), a molecule essential for the prenylation, posttranslational modification, and chaperone of proteins involved in intracellular vesicle trafficking (Gordiyenko et al. 2010). In the retinal pigment epithelium (RPE), REP1 knockdown has been shown to affect the clearance of endocytosed photoreceptor outer segment discs through inhibition of late phagosome–lysosome fusion events in vitro (Gordiyenko et al. 2010). This is likely to form a major mechanism of primary RPE toxicity in choroideremia. Although REP1 is ubiquitously expressed, mutations in *CHM* result in nonsyndromic retinal degeneration, which may be in part explained by compensatory expression of REP2 in other tissues encoded by the *CHML* gene: an X-derived intronless retrogene present on chromosome

Editors: Eyal Banin, Jean Bennett, Jacque L. Duncan, Botond Roska, and José-Alain Sahel
Additional Perspectives on Retinal Disorders: Genetic Approaches to Diagnosis and Treatment available at www.perspectivesinmedicine.org

 doi: 10.1101/cshperspect.a041279
Cite this article as *Cold Spring Harb Perspect Med* 2023;13:a041279

Cold Spring Harbor Perspectives in Medicine
www.perspectivesinmedicine.org

1q42, with a high degree of homology to *REP1* (Seabra 1996).

In ancient Greek, khórion (χόρῐον) denotes membrane or skin with erēmíā (ἐρημίᾱ) denoting a desert, barren wilderness, or otherwise desolate area. Thus, choroideremia was originally named on the presumption that the choroid was the primary site of degeneration based on the characteristic fundoscopic appearances, as first described in 1872 by Ludwig Mauthner (Mauthner 1872). The other historic description for choroideremia was tapetochoroidal dystrophy, which refers to the yellow, tapetal-like reflex observed in patients with advanced disease. Choroideremia was originally considered a developmental and stationary disorder. However, these assumptions—and that of the choroid being the primary site of degeneration—have all since been refuted. Nonetheless, recent evidence suggests mild choroidal thinning on OCT imaging in affected individuals, consistent with a mild developmental abnormality of the choroid (Xue et al. 2016).

The dominant early symptoms of nyctalopia and constricted visual fields relate to involvement of the RPE with functional deficits in the canonical rod visual cycle hosted by the RPE. The cone visual cycle, in contrast, may be regenerated by Müller cells and other RPE-independent mechanisms (Muniz et al. 2007). The genetic basis of choroideremia was identified relatively early through positional cloning in 1990, supported by the characteristic clinical phenotype, disease severity, and X-linked inheritance (Cremers et al. 1990c). Over 350 disease-associated mutations have been described in the *CHM* gene, most of which are null, or presumed functional nulls, resulting in the absence or functional abrogation of REP1 (Han et al. 2021). Given the small size of the *CHM* cDNA (1.96 kb), the slowly progressive nature of choroideremia, easily identifiable clinical phenotype and inheritance pattern, and the absence of amblyopia and/or significant developmental abnormalities, choroideremia has been an ideal candidate for adeno-associated viral (AAV) retinal gene therapy as a therapeutic approach.

This review focuses on the challenges in gaining regulatory approval of retinal gene therapy vectors for choroideremia. To contextualize these discussions, we first summarize the current understanding of clinical phenotype from multimodal retinal imaging studies, and discuss their relevance to the natural history of disease, patient selection for clinical trials, and end points in the assessment of treatment efficacy. Recent findings from pivotal retinal gene therapy clinical trials for choroideremia are summarized (Table 1), followed by a detailed discussion in the challenges toward regulatory approval of the first therapy. In particular, the slow nature of retinal degeneration in choroideremia creates challenges in detecting a benefit over a short pivotal clinical trial. An improvement in best-corrected visual acuity (BCVA) is only possible in patients with advanced disease where efficacy may be limited by RPE dysfunction and degeneration at or around the fovea. Moreover, the effect of surgical detachment of the fovea, necessary for the transduction of the foveal RPE, cannot easily be controlled for in clinical trials. Furthermore, disease-related variability in key outcome measures may obscure therapeutic signals in clinical trials. These challenges can, in part, be overcome and the approval of the first therapy for choroideremia is expected in the near future, 150 years after the first description of the disease.

CLINICAL PHENOTYPE

Affected males with choroideremia present with night blindness in childhood due to the effects of peripheral RPE degeneration on the rod visual cycle and subsequent rod cell death. In early disease, midperipheral retinal degeneration may be identified on clinical examination with retinal thinning and pigment clumping. The visual field becomes progressively constricted as centripetal degeneration of the RPE, retina, and choroid progresses toward the fovea (Fig. 1). Although choroideremia may be clinically misclassified as retinitis pigmentosa (RP) in early disease, as Mauthner observed (Mauthner 1872), the retinal vessels are not constricted and the optic disc is not atrophic due to the associated choroidal degeneration, which prevents retinal hyperoxia and subsequent vaso-

Table 1. Summary of interventional retinal gene therapy clinical trials for choroideremia

Trial	Vector and dose	Phase	Baseline characteristics	Follow-up	Center	References
Nightstar/Biogen NCT01461213 2011–2018	rAAV2.REP1 $0.6–1.0 \times 10^{10}$	1/2	23–89 Early Treatment Diabetic Retinopathy Study (ETDRS) letters 6.4–13.0 db $n = 6$	6 mo	University of Oxford, UK	MacLaren et al. 2014 Noted that patients with advanced choroideremia had the most significant visual acuity gains
	rAAV2.REP1 $0.6–1.0 \times 10^{10}$	1/2	23–89 ETDRS letters 6.4–13.0 db $n = 6$	3.5 yr	University of Oxford, UK	Edwards et al. 2016 Treatment effects sustained to 3.5 yr
	rAAV2.REP1 1.0×10^{10}– 1.0×10^{11}	1/2	23–89 ETDRS letters $n = 14$	2–5 yr	University of Oxford, UK	Xue et al. 2018 Treatment effects sustained to 5 yr, including three study eyes that gained >3 lines of vision (additional data: Simunovic et al. 2016; Xue et al. 2016)
STAR study Nightstar/Biogen NCT03496012 2017–2020	rAAV2.REP 1.0×10^{10} 1.0×10^{11} Untreated	3	34–73 ETDRS $n = 169$	1 yr	International, multicenter 18 centers	Data not published (Biogen press release, Biogen 2021)
GEMINI study Nightstar/Biogen NCT03507686 2017–2022	rAAV2.REP1 1.0×10^{11}	2	$n = 60$ >/= 34 letters	2 yr	International, multicenter six centers	Data not published
University of Oxford REGENERATE NCT02407678 2016–2021	rAAV2.REP1	2	$n = 30$ >/= 34 letters	2 yr	University of Oxford and Moorfields Eye Hospital, UK	Data not published
Nightstar/Biogen SOLSTICE NCT03584165 2018–2027	rAAV2.REP1 rAAV8.RPGR	Follow-up	$n =$ up to 440 Observational long-term follow-up study	5 yr	International, multicenter 18 centers	Data not published

Continued

Table 1. *Continued*

Trial	Vector and dose	Phase	Baseline characteristics	Follow-up	Center	References
THOR study Tübingen NCT02671539 2016–2018	rAAV2.REP1 1×10^{11}	2	$n = 6$ ETDRS 35–75 letters	2 yr	Tübingen, Germany	Fischer et al. 2019 Improvements versus controls over 24 mo in best-corrected visual acuity (BCVA) and microperimetry (MP) but not statistically significant Fischer et al. 2020 After 12-mo, one participant gained 17 letters
Spark Therapeutics NCT02341807 2015–2022	AAV2.hCHM Doses not stated	1/2	$n = 15$ ETDRS > 35 letters Central field <30°	5 yr	USA three sites	Data not published
4D Molecular Therapeutics NCT04483440 2020–2023	Intravitreal 4D-110 AAV.hCHM	1	$n = 15$ ETDRS >/= 34 letters	6 mo	USA two sites	Data not published
Foundation Fighting Blindness, Canada NCT02077361 2015–2025	rAAV2.REP1 (as used in NCT01461213)	1/2	$n = 6$	2 yr	Alberta, Canada	Dimopoulos et al. 2018
Bascom Palmer, Florida NCT02553135 2015–2018	AAV2.REP1 10×10^{11}	2	$n = 6$ ETDRS 35–75 letters	2 yr	Bascom Palmer, Miami, USA	Lam et al. 2019 Supports BCVA as an outcome measure in advanced choroideremia

Cold Spring Harbor Perspectives in Medicine
www.perspectivesinmedicine.org

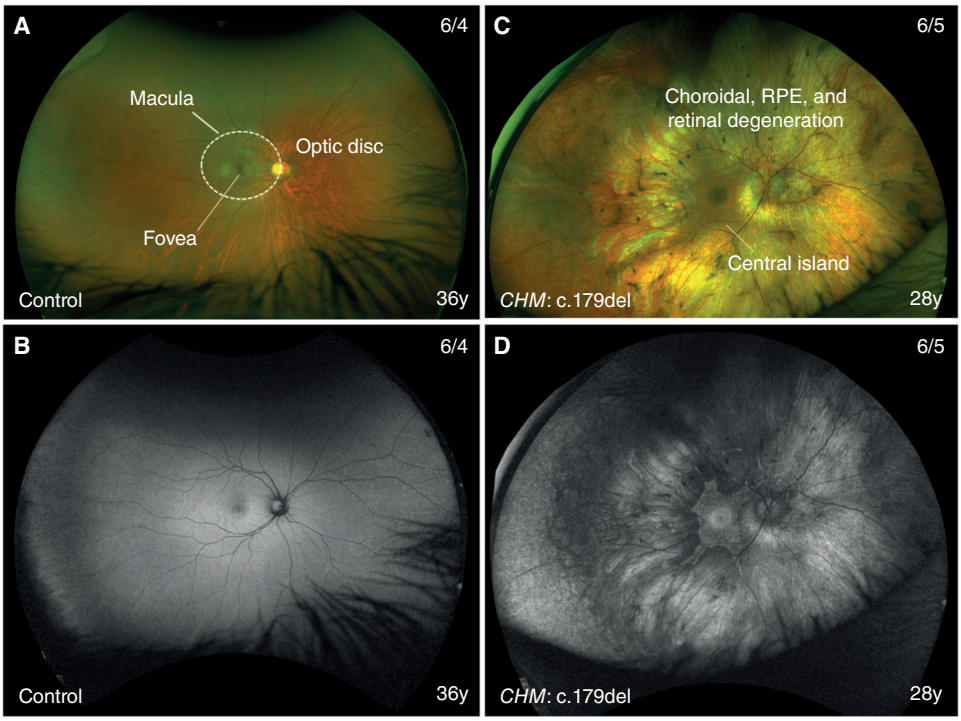

Figure 1. Retinal appearance in choroideremia. (*C*,*D*) A 28-yr-old male with a hemizygous frameshifting mutation in *CHM* (c.179delA) who presented with nyctalopia in childhood. Pseudocolor retinal imaging demonstrates midperipheral RPE, retinal, and choroidal degeneration exposing the pale underlying sclera, and deep choroidal vessels. Centripetal degeneration preserves a central island of retinal pigment epithelium (RPE) and retina; foveal preservation typically sustains an excellent visual acuity until late in the disease. Although pigment migration may be identified in the midperiphery, in contrast to retinitis pigmentosa (RP), choroidal degeneration prevents secondary retinal vascular attenuation. Fundus autofluorescence (FAF) imaging more clearly defines the central island of residual RPE with scalloped edges typical of choroideremia. The fovea is typically threatened from degeneration of the nasal side of the central island (Jolly et al. 2017), which may be due to the temporal displacement of the fovea on the optical axis. (*A*,*B*) A healthy control is shown for comparison. Widefield pseudocolor and autofluorescence images are shown (Optos, Dunfermline, UK).

constriction seen in RP. Optical coherence tomography (OCT) imaging (Fig. 2) reveals RPE loss at the leading edge of the degeneration with loss of outer retina and choriocapillaris thereafter. Absolute scotomas are present in retinal locations where the RPE and retina have degenerated. Typically, by the third decade, a central island of residual retinal tissue with scalloped edges remains, which undergoes exponential decay over time (Aylward et al. 2018). Central visual function, such as BCVA and mesopic microperimetry within the fovea and parafovea are well preserved, or normal, until late in the disease (Figs. 2 and 3). In a proportion of individuals, short-wavelength autofluorescence (SW-AF) imaging demonstrates a mottled RPE surrounding a central smooth zone where photoreceptor morphology is better preserved, validating the mottled zone as a degenerative feature of both the RPE and retina (Stevanovic et al. 2020). Color vision in choroideremia is typically lost along the tritan axis in early disease, as estimated by the Cambridge Color Test, correlating well with visual acuity (Seitz et al. 2018). Encroachment of the degeneration on the fovea is followed by a reduction in visual acuity, with visual decline accelerating between the age of 30 and 40 years (Bozkaya et al. 2022),

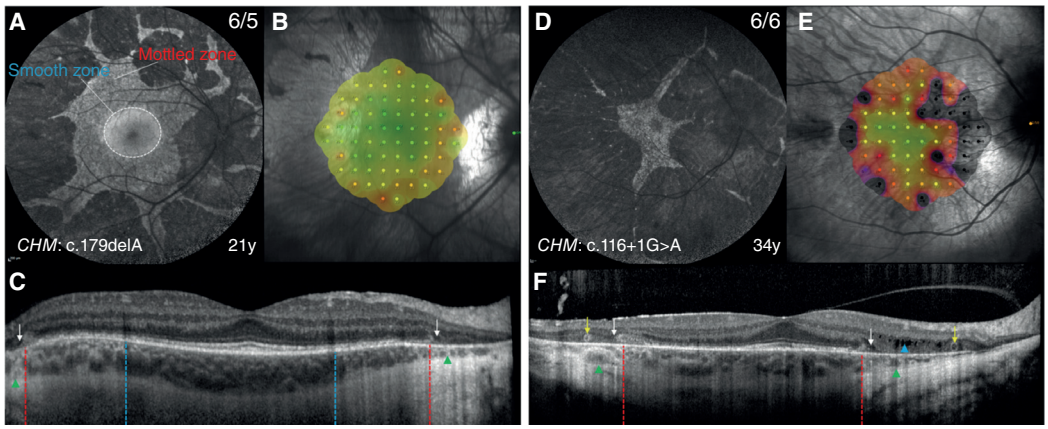

Figure 2. Clinical features of early choroideremia. (*A*) A central island of retinal pigment epithelium (RPE), retina, and choroid typically develops in the third decade in males with a frameshifting mutation in *CHM*, although visual acuity is preserved initially. On short-wavelength autofluorescence (SW-AF) imaging, a mottled zone is observed on the peripheries of the island with a smooth zone in the center in early disease stages. On optical coherence tomography (OCT) imaging, the mottled zone manifests as incomplete choroidal transmission (blue dashed line (*C*) with complete choroidal transmission identifying the peripheral limits of RPE atrophy (red dashed line). Moreover, the ellipsoid zone appears discontinuous over the mottled RPE. Retinal sensitivity falls on the edges of the central island as the degeneration progresses centripetally (*B*). Choroidal and photoreceptor degeneration most likely occur secondary to RPE degeneration, since the choroid (green arrowheads) and photoreceptors (white arrows) are present beyond the limits of RPE atrophy on OCT imaging (*C*,*F*). With advancing disease and a reduction in size of the remaining island, the smooth zone may be lost (*D*) with choroidal transmission seen under the fovea (*F*). Loss of the RPE and overlying retina results in absolute scotomas on microperimetry seen outside of the central island (*E*). Outer retinal tubulations are seen on OCT imaging (yellow arrows, *F*) in the degenerate outer retina. Schisis within the macula (blue arrowhead, *F*) and epiretinal membrane may develop as nonspecific effects of retinal degeneration.

and blindness in the fifth to sixth decade of life. Outer retinal tubulations are present in >90% of individuals (Xue et al. 2016); schisis within the retina is often seen in late disease (Fig. 3).

Although isolated *CHM* mutations result in nonsyndromic retinal degeneration, recent evidence suggests that systemic lipid metabolism may be affected (Cunha et al. 2021). However, it is unclear as to whether serum lipids and cardiovascular disease risk are higher in choroideremia patients compared to age-matched controls.

Differential Diagnosis

For nonsyndromic individuals with a choroideremia-like phenotype without mutations in the *CHM* gene, sequencing of the *RPE65* gene may identify a specific dominant mutation (D477G) that may phenocopy as choroideremia through a presumed dominant-negative disease mechanism (Table 2; Bowne et al. 2011). Individuals in this group typically become symptomatic in early adulthood with nyctalopia and central visual disturbance, with severe visual loss at 40–70 years of age (Hull et al. 2016; Jauregui et al. 2020; Kiang et al. 2020). Heterozygous null mutations in *RGR* and a specific missense mutation in *RHO* have been reported with choroideremia-like phenotypes in separate pedigrees (Audo et al. 2010; Ba-Abbad et al. 2018). Other nonsyndromic differential diagnoses of choroideremia include progressive bifocal chorioretinal atrophy. Nongenetic causes include acquired disease due to extensive peripheral retinal ablative procedures, such as cryotherapy or drug toxicity, such as from thioridazine use.

For individuals with syndromic features and a choroideremia-like retinal phenotype, consideration should be given to a chromosomal deletion involving flanking genes at the *CHM* locus, or a

Cold Spring Harbor Perspectives in Medicine
www.perspectivesinmedicine.org

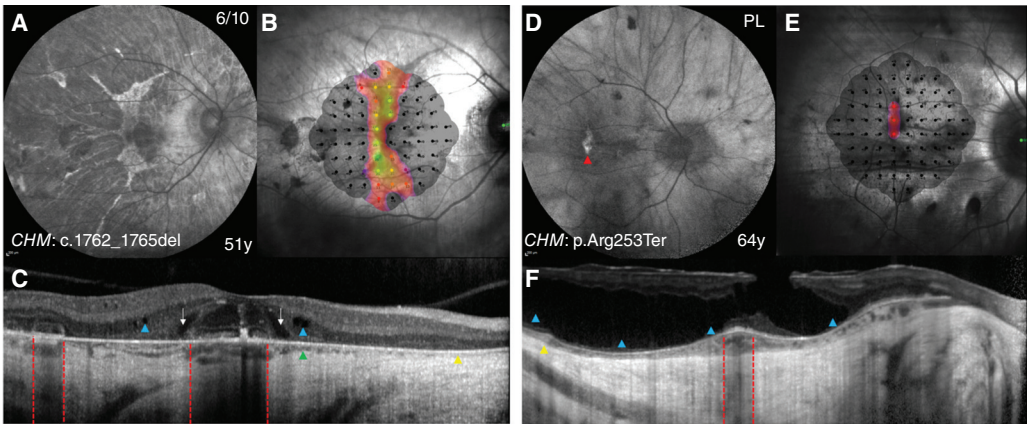

Figure 3. Clinical features of late choroideremia. (*A*,*B*) With advanced disease, the central island degenerates further, associated with a reduction in visual acuity and foveal sensitivity with foveal encroachment. Optical coherence tomography (OCT) and autofluorescence imaging confirm retinal pigment epithelium (RPE) loss (red dashed lines, *C*), and complete choroidal degeneration (yellow arrowhead). Schisis cavities within the retina may progress and extend to the peripheral retina (blue arrowheads, *C*,*F*). (*D*,*E*) In end-stage disease, RPE remnants may remain with perception of light vision and minimal retinal sensitivity before loss of the island results in total blindness.

deletion at the tip of the long arm of the X chromosome. Learning difficulties and deafness are recurrent syndromic features associated with chromosomal deletions involving the *CHM* gene (Cremers et al. 1989; Merry et al. 1989; Hildebrand et al. 2007; Poloschek et al. 2008; Iossa et al. 2015; Liang et al. 2017). Short stature secondary to hypogonadotrophic hypogonadism with ataxia due to cerebellar vermis hypoplasia, spastic paraplegia, and/or learning difficulties should raise suspicions of Oliver–MacFarlane syndrome caused by mutations in *PNPLA6* (Table 2; Kmoch et al. 2015; O'Neil et al. 2019; Wu et al. 2021). A further differential for a syndromic child with a choroideremia-like retinal appearance includes Alagille syndrome, associated with mutations in *JAG1* in 90% of affected individuals. However, severe systemic disease including biliary atresia and congenital heart disease may dominate the clinical picture, particularly as visual acuity is well preserved in early life. A choroideremia-like phenotype may be evident in Danon disease (Taylor and Adler 1993) in which mutations in lysosome-associated membrane protein-2 (*LAMP-2*), expressed in the RPE (Thompson et al. 2016; Fukushima et al. 2020), produce midperipheral pigmentary change associated with hyperreflective foci in the outer nuclear layer on OCT imaging (Hasegawa et al. 2022). *LAMP-2*, present on the X chromosome, is an important constituent of the lysosomal membrane involved in autophagy within the RPE. Systemic manifestations of Danon disease include cardiomyopathy (hypertrophic in males and dilated in females) and generalized myopathy with many patients requiring cardiac transplantation (Taylor and Adler 1993).

Table 2. Differential diagnosis of choroideremia

Nonsyndromic
Gyrate atrophy (*OAT*)
Dominant mutation in *RPE65* (D477G)
Progressive bifocal chorioretinal atrophy (locus: *6q14-16*)
Excessive cryotherapy, or other ablative procedures
Drug toxicity (i.e., thioridazine)
Rhodopsin mutation (M207R) (Audo et al. 2010)
Dominant RGR mutation (haploinsufficiency) (Ba-Abbad et al. 2018)
Syndromic
Deletion on the X chromosome, involving *CHM*
Oliver–MacFarlane syndrome (*PNPLA6*)
Alagille syndrome (*JAG1*)
Danon disease (*LAMP2*)

Cold Spring Harbor Perspectives in Medicine
www.perspectivesinmedicine.org

Female Carriers

Female carriers of choroideremia were recognized long before confirmation of the *CHM* gene on the X chromosome in 1990 in affected individuals. Initial reports noted peripheral retinal pigmentation in female carriers (François 1971) with phenotypic heterogeneity (Forsius et al. 1977). In 1986, a description of 126 female carriers identified symptoms in ~7%, although 40% had visual field changes and 33% abnormal dark adaptation (Kärnä 1986). Full-field electroretinography was found to be affected in ~15% of carriers (Sieving et al. 1986). Disease is slowly progressive in carriers with marked variability, although visual acuity is typically well preserved (Kärnä 1986). FAF imaging may detect subtle mottling of the RPE in the absence of manifest fundoscopic signs (Ortiz-Ramirez et al. 2020), and other multimodal retinal imaging techniques may be supportive (Ma et al. 2017; Paavo et al. 2019; Corvi et al. 2020; Murro et al. 2020).

Four main carrier phenotypes are recognized based on multimodal retinal imaging findings: Fine, Coarse, Geographic, and Male-pattern (Fig. 4; De Silva et al. 2021). The variable clinical

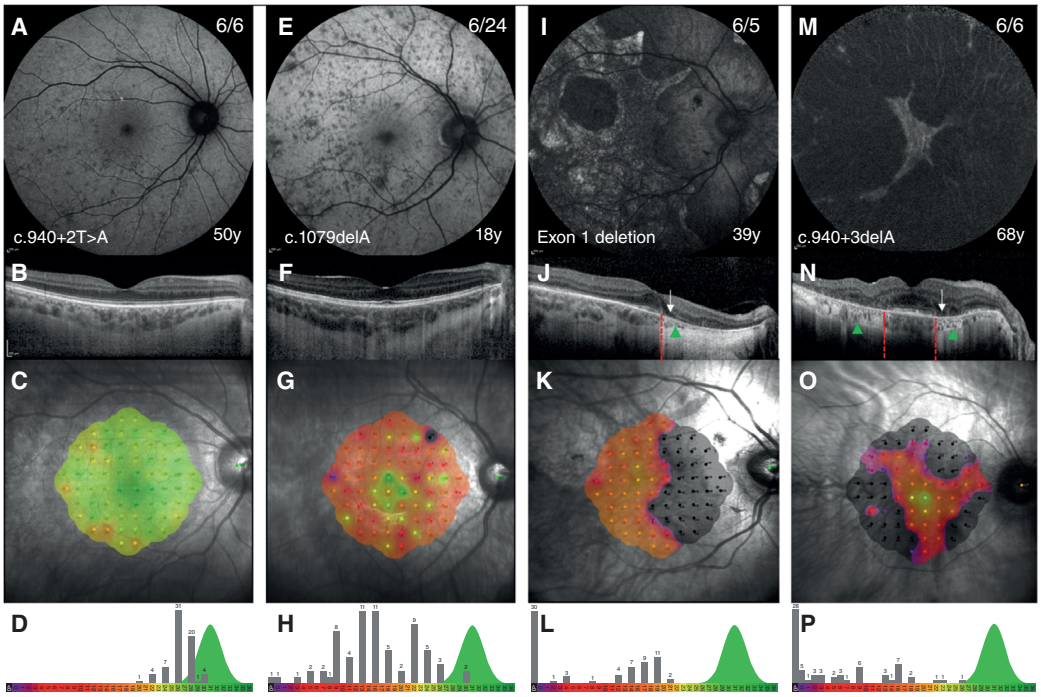

Figure 4. Multimodal retinal imaging features in four female *CHM* carriers. The retinal phenotype in female carriers of *CHM* mutations is dependent on the embryonic stage and the skew of X-inactivation, the specific variant, age, and other modifiers. (*A–D*) Fine, (*E–H*) Coarse, (*I–L*) Geographic, and (*M–P*) Male pattern choroideremia carrier phenotypes are described. Initially, fine patchy (*A*) hypoautofluorescence may be identified with a normal appearance of the retinal layers on OCT. An individual with a coarse mottled pattern shows streaks of choroidal hypertransmission on OCT imaging (*F*), suggesting patchy disease of the RPE, and reduced retinal sensitivity (*G–H*). With progressive disease, focal loss of RPE results in geographic atrophy, characterized by loss of autofluorescent signal (*I*), choroidal hypertransmission on OCT imaging (*J*, dashed red line), and absolute scotoma on microperimetry (*K–L*). Note that preserved retina has a coarse appearance and reduced function. Further degeneration may result in a male phenotype with an island of residual RPE at the posterior pole with overlying retina (*N*) that may be sufficient to support a visual acuity of 6/6 if the fovea is preserved. As in affected males, the choroid (green arrowheads) and retina (white arrow) is preserved beyond the area of remaining RPE.

Cold Spring Harbor Perspectives in Medicine
www.perspectivesinmedicine.org

phenotypes are determined by the stage and pattern of X chromosomal inactivation that occurs in mammals through heterochromatin packaging, as described originally by Mary Lyons (Lyon 1961). In human embryonic development, X-inactivation occurs around the blastocyst stage, and remains fixed throughout the life of the cell and all descendant cells. In female carriers, skewed inactivation of the X chromosome containing the wild-type *CHM* allele in the early blastocyst stage may result in severe, male pattern disease, since most or all RPE cells are affected. Later inactivation may result in a geographic pattern where localized clonal expansion of affected RPE cells manifests clinically in coalescing patches of RPE atrophy. A fine or coarse granular or mottled appearance on FAF imaging may represent later X-inactivation, whereby normal and abnormal RPE cells are juxtaposed throughout the retina. Moreover, different patterns of X-inactivation between RPE lineages may underpin interocular asymmetry in carrier females. Accordingly, while some carrier females remain asymptomatic, others may exhibit a more severe disease phenotype similar to that observed in affected males (Jauregui et al. 2019). Note that these patterns are distinct from carriers of X-linked RP where radial streaks are apparent from migration of affected and unaffected photoreceptors during development, and X-linked albinism where a "mud-splattered" fundus secondary to mosaicism of the melanosomes in affected and unaffected RPE cells is observed with radial streaks in the periphery (Wu et al. 2018). Bruch's membrane degeneration in a choroideremia carrier may be complicated by choroidal neovascularization (Wavre-Shapton et al. 2013; Ang et al. 2021).

Although mosaicism of the X chromosome may be determined from peripheral white blood cells, the cell lineages are distinct from those relevant to X-linked inherited degenerations, which affect the retina and RPE. It has been assumed that skewed X-inactivation (>80% inactivation of the healthy X chromosome) (Ørstavik 2006) may lead to male pattern phenotypes of X-linked diseases. However, a study of 12 female carriers of disease-associated *CHM* alleles found no correlation between disease severity and skewed lyonization of the wild-type *CHM* allele (Perez-Cano et al. 2009). These assays are complicated by age-dependent skewed X-inactivation in peripheral blood, which increases after the age of 55 years (Ørstavik 2006). An extreme demonstration of severe, male pattern choroideremia in a female carrier has been reported in the context of Turner's syndrome—monosomy of the X chromosome in females (Cheng et al. 2018).

CLINICAL GENETICS

The familial nature and male predominance of choroideremia was appreciated from early reports, with X-linked inheritance suggested in 1942 by Goedbloed (Goedbloed 1942). The locus harboring the *CHM* gene was provisionally assigned to Xq13-21 (Lewis et al. 1985; Nussbaum et al. 1985; Schwartz et al. 1986), and further narrowed thereafter to Xq21 (Cremers et al. 1987, 1989; Nussbaum et al. 1987; Sankila et al. 1990) with the underlying *CHM* gene identified as the genetic cause of choroideremia (Xq21.2) through positioning cloning in 1990 (Cremers et al. 1990c). The *CHM* gene encodes REP1, a protein of 653 amino acids that is ubiquitously expressed in human tissues. More than 350 mutations in *CHM* have been identified (as summarized in detail by Han et al. 2021), all of which are presumed to abrogate or reduce the biological function of REP1, either due to reduced expression and/or reduced chaperone or prenylation activity. Most exonic mutations are truncating with only six missense mutations presumed to act as functional nulls (Han et al. 2021). The low number of missense mutations in *CHM* is striking and supports the known primary function of REP1 as a chaperone protein, whose function can be compensated for in nonocular tissues by REP2 with a 95% sequence homology. Numerous intronic mutations and large deletions have been identified, together accounting for ~33% of pathogenic variants (Han et al. 2021). The large number of reported deletions in *CHM* may be explained by a weak selection pressure due to compensation in other tissues by REP2 and the relatively late onset of end-stage disease in affected males (i.e., after the age of reproduction). Molecular genetic testing

is typically undertaken through next-generation sequencing of exons and intron–exon boundaries. However, several pathogenic deep intronic mutations have been identified, which may require direct sequencing of the gene if the clinical suspicion is high. Moreover, the detection of large deletions, insertions, duplications, and rearrangements may require multiplex ligation-dependent probe amplification (MLPA), which together may account for as many as 14% of mutations (Han et al. 2021). Additional variants have been described affecting highly conserved sequences within the *CHM* promoter (Vaché et al. 2019). Since most pathological mutations in *CHM* are functional nulls, there is only limited evidence for correlation between genotype and phenotype (Simunovic et al. 2016). The large number of unique variants in *CHM* further supports gene therapy as the leading therapeutic strategy. Other strategies are reviewed in detail elsewhere (Cehajic Kapetanovic et al. 2019a,b; Han et al. 2021).

DISEASE MECHANISM

CHM encodes REP1, which functions in the posttranslational modification of proteins through prenylation, a process by which covalent lipid (hydrophobic) prenyl molecules are attached to the carboxyl terminus of a protein to facilitate attachment to intracellular membranes (Fig. 5). Specifically, REP1 facilitates the transfer of a geranylgeranyl group by GGTases to Rab GTPases, a superfamily of Ras G-proteins involved in intracellular vesicle trafficking. The absence of REP1 has been associated with the re-

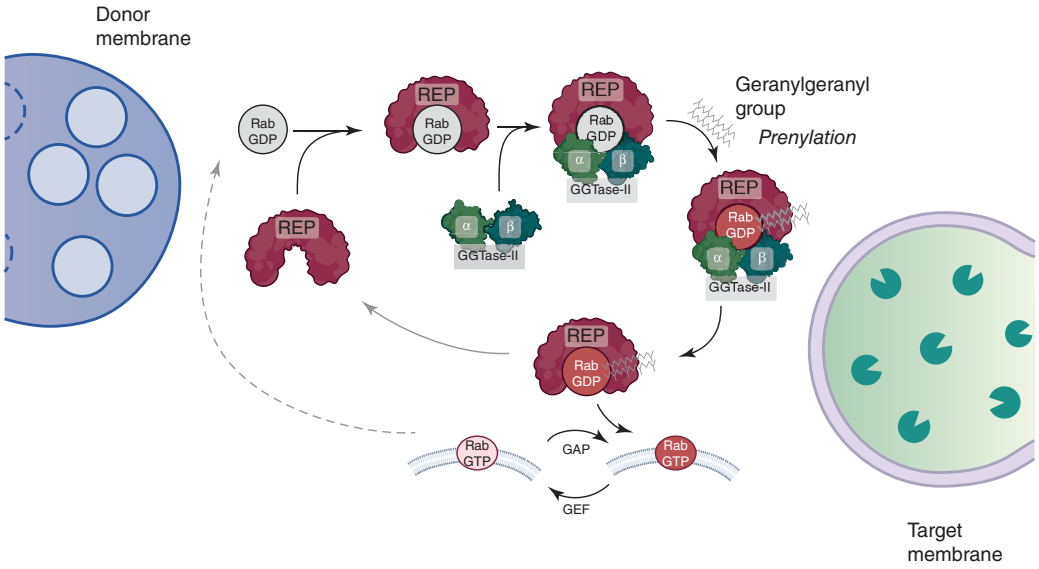

Figure 5. REP1 function within the retinal pigment epithelium (RPE). The *CHM* gene encodes REP1, which functions as a geranylgeranyl transferase in the prenylation posttranslational modification of Rab proteins, escorting them to their cellular targets. Prenyl molecules are attached to the carboxyl-terminus Rab proteins, facilitating their attachment to cell membranes. The REP cycle shows the escort of Rab GDP by REP1, the addition of α- and β-subunits through GGTase-II, and the addition of a geranylgeranyl group to the carboxyl terminus of the Rab protein (prenylation), which facilitates membrane attachment. In the RPE, shed outer segment discs from photoreceptors are continually phagocytosed by the RPE, fused to endosomes to form the late phagosome, and later fused with lysosomes to form the phagolysosome toward the basolateral RPE membrane where a low pH facilitates degradation. The ablation of REP1 has been shown to delay phagosome maturation and clearance of photoreceptor outer segments, with accumulation of debris within the RPE, which is the probable mechanism underlying primary RPE cell death in choroideremia. In the retina, REP1 is known to interact primarily with Rab27a and others to facilitate prenylation, which in turn coordinates inward movement of the phagolysosome.

Cold Spring Harbor Perspectives in Medicine
www.perspectivesinmedicine.org

duced prenylation of several Rabs (Seabra et al. 1995). In addition, REP1 is responsible for escorting Rab proteins through the cytoplasm to their target membrane.

In the RPE, efficient phagocytosis of shed photoreceptor outer segments is critical for the maintenance of homeostasis. The Rab family of GTPases is involved in phagosome maturation within the RPE, whereby phagocytosed outer segments fuse with endosomes and lysosomes to form mature phagolysosomes (Kwon and Freeman 2020). Two independent studies identified RPE dysfunction and degeneration following ablation or silencing of *CHM* associated with alterations in phagocytic and secretory pathways within the RPE (Gordiyenko et al. 2010; Wavre-Shapton et al. 2013). Furthermore, restoration of the human transgene to *CHM*-deficient iPSC-derived RPE cells restored prenylation, vesicle trafficking, and phagocytosis (Duong et al. 2018). Multimodal imaging studies in affected patients (Figs. 2 and 3) show RPE atrophy at the leading edge of degeneration, followed thereafter by loss of the outer retina and choriocapillaris. However, the pathophysiology of disease is likely to be more complex. Conditional knockout models using inducible and tissue-specific Cre expression showed independent degeneration of RPE and photoreceptors that involved different subsets of Rab proteins (Tolmachova et al. 2006). Independent degeneration of photoreceptors and early loss of choriocapillaris perfusion within preserved RPE have also been observed in choroideremia patients (Jacobson et al. 2006; Foote et al. 2019). Loss of the rod photoreceptor outer segments prior to RPE loss may in part explain early loss of rod function observed in patients with choroideremia (Aleman et al. 2017).

Although there are observations of photoreceptor defects in animal models that appear independent of RPE degeneration (Tolmachova et al. 2006), overall, the evidence supports the RPE as the most likely primary site of degeneration in choroideremia. Delays in phagosome maturation and photoreceptor outer segment clearance with consequent accumulation of debris within the RPE are the most likely pathogenic mechanisms for cellular toxicity, although others, such as deficits in melanosome trafficking have been suggested (Hume et al. 2001). Other proteins involved in the prenylation pathway do not appear to influence disease progression in affected individuals when evaluated ex vivo (e.g., *CHML*, *RABGGTB*, *RAB27A*), nor do their levels of expression differ from controls (Fry et al. 2021). However, prenylation of Rabs (e.g., RAB27A and RAB6A) may be assessed in vitro as a functional assay for assessing the potency of AAV vectors, regardless of the specific *CHM* mutation (Patrício et al. 2018).

REP1 is ubiquitously expressed and, in nonhuman primates, its absence is embryonically lethal (Tolmachova et al. 2006; Moosajee et al. 2009). It is unclear why mutations in *CHM* result in nonsyndromic retinal degeneration. However, in humans, the X-derived retrogene, REP2, presents as cDNA (i.e., without introns) on chromosome 1 as the *CHML* gene bears a 95% sequence homology to REP1 and may provide a compensatory mechanism in nonocular tissues facilitated by other Rabs (Cremers et al. 1994). Rab27a is preferentially prenylated by REP1 and is particularly important for retinal function (Larijani et al. 2003). However, it is unclear whether nonsyndromic retinal degeneration occurs due to altered activity of specific Rab(s), or whether the RPE is particularly susceptible to long-term abnormalities of intracellular vesicle trafficking.

RPE degeneration appears to affect the midperipheral retina early in choroideremia. This observation may be explained by the relatively high metabolic demands on the RPE in the midperipheral retina, which forms a watershed zone in which the relative density of photoreceptors to RPE cells is highest across the retina (5000 RPE cells per mm^2 at the fovea; 2500 per mm^2 in the peripheral retina). In choroideremia and other primary RPE diseases, such as gyrate atrophy, the midperipheral RPE and retina is affected first, followed by progressive RPE degeneration, which then progresses centripetally and contiguously. This observation might also explain why the foveal encroachment in late disease occurs initially on the nasal side of the island (Figs. 2 and 4–6).

CLINICAL TRIALS

Table 1 lists the interventional clinical trials of retinal gene therapy for choroideremia, with a summary of study characteristics, key findings, and references to published data. The most advanced clinical program is the STAR study (NightStaRx/Biogen; NCT03496012), a pivotal international phase 3 clinical trial, which evaluated the effect of subretinal AAV2.REP1 over 12 mo in individuals with advanced choroideremia (34–73 ETDRS letters). Although primary and secondary end points were not met in this study (Biogen press release, Biogen 2021), a trend to clinical benefit aligned in the treatment group across all end points, narrowly missing statistical significance following Bonferroni correction. However, there is as yet nothing in the public domain to suggest that the sponsor is intending to negotiate with the U.S. FDA on whether the marginal clinical data might be sufficient to justify some form of limited approval. It is likely that a further phase 3 clinical trial will be undertaken, which may benefit from observations made during the STAR study, including refinements in study design, such as vector dose, surgical technique, and consideration of novel end points. These modifications may help to overcome the challenges in gaining regulatory approval of retinal gene therapy for choroideremia, which will be considered in detail.

CHALLENGES IN THE REGULATORY APPROVAL OF GENE THERAPY FOR CHOROIDEREMIA

The principal challenges in the approval of the first gene therapy for choroideremia are (1) the short duration of pivotal clinical trials for a very slow degeneration; (2) a requirement for visual acuity improvement necessitates the inclusion of patients with late disease because this is when the acuity begins to drop; (3) the need for surgical detachment of the fovea for transduction of the subfoveal RPE—a process that in itself will have a detrimental effect on visual acuity that is not seen in noninterventional controls; and (4) the transduction of dysfunctional RPE cells in patients with late disease. These challenges are a consequence, primarily, of the thresholds required to satisfy regulators and the costs of running clinical trials. In preclinical studies, which are not subject to the same constraints, retinal gene therapy, in general, has been shown to be most effective when delivered in the early stages of disease when a greater number of target cells are available, with therapeutic efficacy demonstrated over a relatively longer period.

Short Duration of Clinical Trials

Inherited retinal degenerations are individually rare, often slowly progressive, and may have ocular asymmetry. These characteristics alone present challenges in the demonstration of benefit during short pivotal clinical trials, which are typically 1–2 years in duration, given that degeneration in the control (untreated) eyes is slow. This often means that sustained improvement of visual function in a proportion of participants is necessary to show a clinically and statistically significant difference between treatment groups for regulatory approval. For retinal gene therapy, which for the most part aims to prevent further visual loss through the prevention of cell death of transduced target cells, this is a somewhat burdensome objective. Moreover, visual improvement may not be possible for all genetic causes of retinal degeneration, which vary in terms of the primary affected cell type, the type of protein (e.g., visual cycle, structural, ciliary, phototransduction), the speed of retinal degeneration, and age of onset. The length of clinical trials in retinal gene therapy is generally restricted due to the associated costs. However, a conditional approval enabling longer-term observations prior to final approval may circumvent the challenge with evaluation of gene therapy for slow retinal degenerations, as discussed in more detail below.

The Inclusion of Patients with Late Choroideremia

The central, key functional outcome measure in clinical trials in ophthalmology remains BCVA,

Cold Spring Harbor Perspectives in Medicine
www.perspectivesinmedicine.org

with the degree of improvement defined in terms of letter or lines of improvement on the ETDRS chart. These outcome measures have become standard in the assessment of efficacy of interventions (e.g., anti–vascular endothelial growth factor [VEGF] injections) for the major acquired macular disorders (e.g., age-related macular degeneration, diabetic macular edema) where significant, short-term visual benefits are expected. However, the pathobiology and natural history of these late-onset acquired disorders are distinct from inherited retinal degenerations, as are the therapies used to address them. Moreover, BCVA represents a high-contrast stimulus, which is an imperfect measure of a patient's visual function. For the Nightstar/Biogen STAR phase 3 clinical trial, the threshold for approval predesignated by the FDA as the primary end point was a three-line (15-letter) gain in a significantly greater proportion of patients compared with the control group. Choroideremia patients (Figs. 2 and 3) typically maintain visual acuity until late in the disease. Therefore, gains in BCVA require the inclusion of late-stage patients whose central vision has dropped to between 35–73 ETDRS letters (to allow headroom to gain 15 letters—88 letters is better than 6/6 or 20/20). Conversely, below 35 letters (6/60 or 20/200), fixation becomes unreliable and visual acuity readings become inconsistent. This group typically exhibits significant central RPE dysfunction and degeneration—the target cell for transduction in choroideremia (such as the patient in Fig. 3)—which may not be optimal for effective transduction. The inclusion of patients with late disease is therefore a consequence of the current accepted regulatory practice, rather than what is generally accepted as optimal in the field of retinal gene therapy—that is, to treat early in the disease process to maximize the therapeutic effect.

Surgical Detachment of the Fovea

Since BCVA is the primary outcome measure in most clinical trials, transduction of foveal cones is a key strategy in retinal gene therapy that often necessitates surgical detachment of the fovea following subretinal injection. The visual thresholds for approval must be achieved despite any adverse structural and functional effects related to the maneuver—an effect that cannot easily be controlled for in clinical trials of subretinal gene therapy due to the associated surgical risks. Surgical detachment of photoreceptors may lead to short-term structural and functional changes within the retina as seen in animal models (Kyhn et al. 2008; Secondi et al. 2012) and in human patients treated with gene therapy (Aleman et al. 2022), which may offset early therapeutic signals in clinical trials. While mild foveal dysfunction with a loss of a few letters of visual acuity may be perfectly acceptable in the context of giving a sight-saving gene therapy treatment, this becomes an issue when the visual acuity gains being chased in a pivotal trial are only marginal. Therefore, in the early postoperative period, retinal function in the region of the bleb/retinal detachment may be worse than measured at baseline. Moreover, focal loss of the RPE is typically apparent at retinotomy sites following the delivery subretinal gene therapy, an effect that should be compensated for in relevant methods of analysis when compared to control eyes (e.g., microperimetry and FAF) across short trials. Consequently, treated eyes must achieve greater effective functional improvements to compensate for the effect of retinal detachment in the absence of a paired control intervention. Further research may help to quantify the structural and functional effects of early surgical retinal detachment within the bleb margin, although this may vary depending on the underlying gene. For subretinal gene therapy, these further data may support the modification of thresholds for approval by the regulators to account for these effects.

RPE Dysfunction in Late Disease

Individuals with late choroideremia are preferentially selected for clinical trials (Table 1) to avoid a ceiling effect that prevents measurable improvements in BCVA. At this late stage, the residual island of RPE is usually mottled, with or without a central smooth zone on FAF imaging (Fig. 3). Mottled RPE has been further

Cold Spring Harbor Perspectives in Medicine
www.perspectivesinmedicine.org

associated with photoreceptor abnormalities on OCT imaging (Stevanovic et al. 2020). These observations support structural degeneration of the remaining RPE in late choroideremia, the primary target cell in retinal gene therapy. It is unclear whether dysfunctional RPE in late choroideremia can be transduced as efficiently as the RPE in early disease or within the smooth zone. In preclinical studies, carrier female mice ($Chm^{-/WT}$) were typically injected at 3–4 wk, without assessments of the effects on REP1 expression following AAV2. REP1 injection in later disease (Tolmachova et al. 2013). Mechanisms of RPE cell death in choroideremia may be REP1-independent in later stages, and therefore not retrievable by REP1 transgene replacement. Furthermore, dosing may be more challenging in late disease where the effective multiplicity of infection (MOI) may be high as fewer remaining, dysfunctional RPE cells remain, which receive a high dose of AAV. The late application of gene therapy may therefore affect the predictability and efficiency of transduction. There are no direct in vitro studies comparing the efficiency of AAV transduction in RPE cell lines with or without degenerative features or toxic insults. However, a comparison of the relative efficacy of retinal gene therapy in preserving vision in subjects with early disease versus late disease may help to answer this question.

Gene therapy for choroideremia aims to restore REP1 function and prenylation activity to the remaining RPE cells, thereby preventing or slowing RPE cell death, and secondary photoreceptor and choroidal degeneration. Substantial improvements in visual acuity in later-stage choroideremia patients suggest that RPE function is improved following subfoveal retinal gene therapy, where it is presumed that restoration of RPE prenylation and intracellular vesicle trafficking improves the function of foveal cones. It is unclear whether the dysfunctional RPE is able to express the REP1 transgene as efficiently. Consequently, the extent to which gene therapy can rescue dysfunctional RPE within the remaining central island of residual tissue is unclear, and whether this varies between the smooth and mottled zones on FAF imaging.

TOWARD REGULATORY APPROVAL

Novel End Points

The FDA emphasizes the importance of functional benefits for the approval of gene therapy vectors for inherited retinal degeneration. Although BCVA is the most recognized functional outcome measure, improvements in BCVA are not always possible in IRDs: this may relate to amblyopia, and/or photoreceptor or RPE degeneration at the fovea. In choroideremia, visual acuity is lost in late disease and the aim of gene therapy is early intervention to preserve the maximum visual function over the long term. In early choroideremia, visual acuity is well preserved (Fig. 2), and a ceiling effect means that alternative functional end points must be sought for clinical trials. Since rod function is lost early in the disease course, end points that evaluate rod function may be supportive. Scotopic microperimetry is a novel end point that measures differential rod and cone sensitivities within the macula—typically targeted in subretinal gene therapy vector delivery (Taylor et al. 2022). In the pivotal clinical trial of *RPE65* gene therapy, the multiluminance mobility test (MLMT) was used as a primary outcome measure that was accepted by the FDA in the process of regulatory approval. However, the MLMT is not relevant to many other retinal degenerations, which may not affect the visual cycle with a profound effect on scotopic visual function. Microperimetry is a key functional outcome measure in many retinal gene therapy clinical trials, permitting a point-wise assessment of macular sensitivity (Pfau et al. 2021). However, in choroideremia, the decay of the central island may lead to precipitous drops in measured retinal sensitivity. Moreover, the retinal locations analyzed on repeat assessment are not perfectly consistent, which may lead to variability (i.e., when retinal function is assessed over a blood vessel). A key technique for development is the accurate mapping of microperimetry plots so that sequential assessments test identical retinal locations and to link microperimetry assessments to structural retinal imaging techniques, such as FAF imaging, to reduce variability between assessments and subjects.

Cold Spring Harbor Perspectives in Medicine
www.perspectivesinmedicine.org

A qualitative difference in the RPE has been noted in the central island in the majority of patients with choroideremia on SW-AF imaging (Stevanovic et al. 2020). Centripetal degeneration results in a mottled zone of degenerating RPE with a more preserved smooth zone centrally with healthier appearing RPE (Fig. 2). A significant difference in the continuity of the ellipsoid zone was observed in mottled versus smooth zone on SW-AF imaging, suggesting indirectly that photoreceptor degeneration is more pronounced over the mottled zone. This suggests the possibility of using the smooth RPE zone (e.g., changes in size and/or retinal sensitivity) as an outcome measure following retinal gene therapy. The objective definition of the smooth and mottled zones on FAF may be further aided by near-infrared autofluorescence (NIR-AF) imaging since the area of the NIR-AF signal appears to broadly match that of the smooth zone on SW-AF imaging (Fig. 6), although this requires further validation. In support of this suggestion, choroideremia patients may be categorized based on NIR-AF patterns, which appear to correlate with other structural measures of degeneration on OCT imaging (Birtel et al. 2019).

Modification of the Process of Regulatory Approval

In general, the difficulty in gaining approvals for novel therapeutics for inherited retinal degenerations by the regulators (e.g., FDA) may necessitate reconsideration of the pathway to regulatory approval that recognizes the slowly progressive nature of IRDs. Pivotal clinical trials are expensive to run; 3–5-year studies would be financially prohibitive, although far more likely to demonstrate the requisite functional benefits. An alternative pathway to approval may be that if safety is demonstrated in a phase 3 study over 1–2 years, with trends toward functional benefit, a period of conditional approval could be granted by the regulators, allowing sponsors to collect

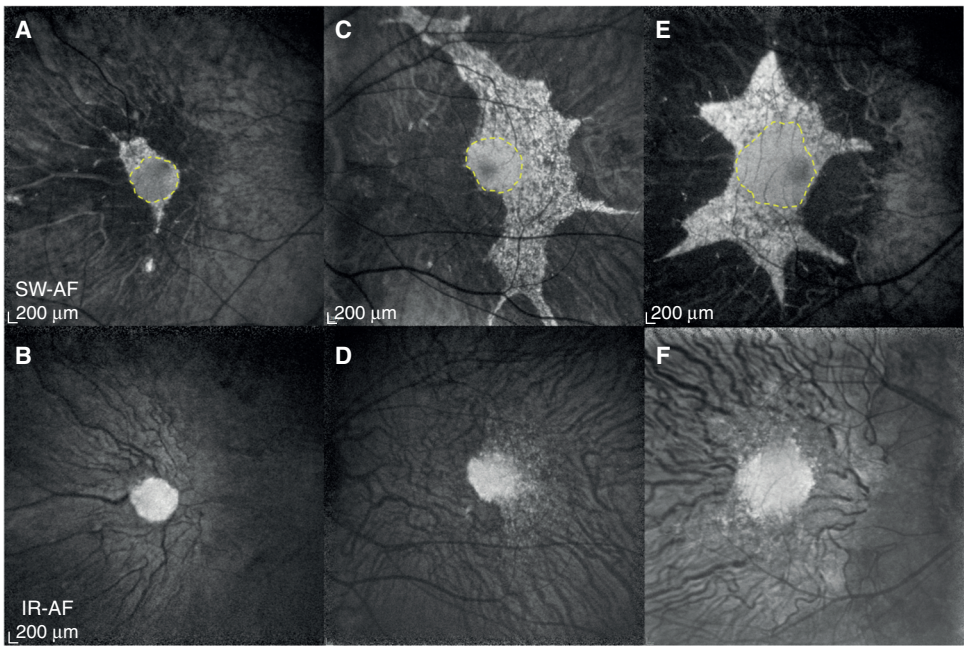

Figure 6. Near-infrared fluorescence imaging in choroideremia. (*A*,*C*,*E*) The smooth zone on short-wavelength autofluorescence maps to residual signal on near-infrared autofluorescence imaging in three patients with choroideremia. (*B*,*D*,*F*) Note that the limits of the central zone on infrared autofluorescence (IR-AF) correspond closely to the approximate boundaries of the smooth zone (yellow dashed line) as seen on short-wavelength autofluorescence (SW-AF) imaging.

longer-term data that can then be reviewed by the regulators prior to final approval.

This approach may change the paradigm of patient selection for clinical trials across different forms of retinal degeneration. In the case of choroideremia, this change would support the inclusion of patients with early disease who have a greater number of target RPE cells and may have a better long-term prognosis following intervention. The effective MOI would be more predictable on early-stage patients, potentially reducing variability between subjects. In late disease, the rescue of dysfunctional target cells may result in an improvement in visual function that typically plateaus once nonoptimally transduced cells, which may contribute to retinal function in the early postoperative period, die. In early disease, this phenomenon may be relevant only to RPE cells at the edge of the remaining island. A clear therapeutic effect could be shown through preservation of the island while the central island in the control eye undergoes exponential decay (Aylward et al. 2018). The current regulatory prerequisites encourage investigators to select patients and doses based on what might show the greatest functional benefit over the shorter term, so that other groups may benefit following approval. However, these may not be the same groups that would otherwise be chosen for clinical trial participation if long-term preservation of vision was the goal. Reform of the process of regulatory approval of gene therapy vectors may benefit patients by focusing patient selection around long-term functional improvements, which would probably be favored by patient groups.

CONCLUDING REMARKS

Choroideremia is an untreatable blinding disease due to mutations in the *CHM* gene. Significant advances have been made in describing the genetic basis and molecular pathophysiology of the disease, with more than 350 mutations described. A pivotal phase 3 clinical trial of retinal gene therapy for choroideremia showed clear therapeutic benefits, although it fell short of the thresholds for regulatory approval. In this review, we summarize the challenges in gaining approval for gene therapy investigational medical products for slow inherited retinal degenerations such as choroideremia and suggest novel end points that might be supportive. Despite these challenges, the approval of the first therapy for choroideremia is expected in the near future, 150 years after the first clinical description of the disease by Mauthner in 1872.

COMPETING INTEREST STATEMENT

R.E.M. is a named inventor on a patent for choroideremia gene therapy owned by the University of Oxford.

ACKNOWLEDGMENTS

R.E.M. is a consultant to Biogen Inc.

REFERENCES

Aleman TS, Han G, Serrano LW, Fuerst NM, Charlson ES, Pearson DJ, Chung DC, Traband A, Pan W, Ying GS, et al. 2017. Natural history of the central structural abnormalities in choroideremia: a prospective cross-sectional study. *Ophthalmology* **124:** 359–373. doi:10.1016/j.ophtha.2016.10.022

Aleman TS, Huckfeldt RM, Serrano LW, Pearson DJ, Vergilio GK, McCague S, Marshall KA, Ashtari M, Doan TM, Weigel-DiFranco CA, et al. 2022. Adeno-associated virus serotype 2-hCHM subretinal delivery to the macula in choroideremia: two-year interim results of an ongoing phase I/II gene therapy trial. *Ophthalmology* **129:** 1177–1191. doi:10.1016/j.ophtha.2022.06.006

Ang JL, Wright AF, Dhillon B, Cackett P. 2021. Choroidal neovascularisation in a predicted female choroideraemia carrier treated with intravitreal anti-vascular endothelial growth factor. *Euro J Ophthalmol* **31:** 4–10. doi:10.1177/1120672120965495

Audo I, Friedrich A, Mohand-Saïd S, Lancelot ME, Antonio A, Moskova-Doumanova V, Poch O, Bhattacharya S, Sahel JA, Zeitz C. 2010. An unusual retinal phenotype associated with a novel mutation in RHO. *Arch Ophthal* **128:** 1036–1045. doi:10.1001/archophthalmol.2010.162

Aylward JW, Xue K, Patrício MI, Jolly JK, Wood JC, Brett J, Jasani KM, MacLaren RE. 2018. Retinal degeneration in choroideremia follows an exponential decay function. *Ophthalmology* **125:** 1122–1124. doi:10.1016/j.ophtha.2018.02.004

Ba-Abbad R, Leys M, Wang X, Chakarova C, Waseem N, Carss KJ, Raymond FL, Bujakowska KM, Pierce EA, Mahroo OA, et al. 2018. Clinical features of a retinopathy associated with a dominant allele of the *RGR* gene. *Invest Ophthalmol Vis Sci* **59:** 4812–4820. doi:10.1167/iovs.18-25061

Biogen. 2021. Biogen announces topline results from Phase 3 Gene Therapy Study in Choroideremia. https://investors.biogen.com/news-releases/news-release-details/biogen-announces-topline-results-phase-3-gene-therapy-study

Cold Spring Harbor Perspectives in Medicine
www.perspectivesinmedicine.org

Birtel J, Salvetti AP, Jolly JK, Xue K, Gliem M, Müller PL, Holz FG, MacLaren RE, Charbel Issa P. 2019. Near-infrared autofluorescence in choroideremia: anatomic and functional correlations. *Arch Ophthal* **199:** 19–27.

Bowne SJ, Humphries MM, Sullivan LS, Kenna PF, Tam LC, Kiang AS, Campbell M, Weinstock GM, Koboldt DC, Ding L, et al. 2011. A dominant mutation in RPE65 identified by whole-exome sequencing causes retinitis pigmentosa with choroidal involvement. *Eur J Hum Genet* **19:** 1074–1081. doi:10.1038/ejhg.2011.86

Bozkaya D, Zou H, Lu C, Tsao NW, Lam BL. 2022. Bilateral visual acuity decline in males with choroideremia: a pooled, cross-sectional meta-analysis. *BMC Ophthalmol* **22:** 29. doi:10.1186/s12886-022-02250-z

Cehajic Kapetanovic J, Barnard AR, MacLaren RE. 2019a. Molecular therapies for choroideremia. *Genes (Basel)* **10:** 738. doi:10.3390/genes10100738

Cehajic Kapetanovic J, Patrício MI, MacLaren RE. 2019b. Progress in the development of novel therapies for choroideremia. *Expert Rev Ophthalmol* **14:** 277–285. doi:10.1080/17469899.2019.1699406

Cheng JL, Farnsworth K, Bernstein PS. 2018. Choroideremia in a woman with turner syndrome. *JAMA Ophthalmol* **136:** 1076–1078. doi:10.1001/jamaophthalmol.2018.2630

Corvi F, Corradetti G, Wong A, Eng JG, Sadda S. 2022. Peripheral optical coherence tomography findings in a choroideremia carrier. *Retin Cases Brief Rep* **16:** 766–769. doi:10.1097/ICB.0000000000001109

Cremers FP, Brunsmann F, van de Pol TJ, Pawlowitzki IH, Paulsen K, Wieringa B, Ropers HH. 1987. Deletion of the DXS165 locus in patients with classical choroideremia. *Clin Genet* **32:** 421–423. doi:10.1111/j.1399-0004.1987.tb03166.x

Cremers FP, van de Pol DJ, Diergaarde PJ, Wieringa B, Nussbaum RL, Schwartz M, Ropers HH. 1989. Physical fine mapping of the choroideremia locus using Xq21 deletions associated with complex syndromes. *Genomics* **4:** 41–46. doi:10.1016/0888-7543(89)90312-1

Cremers FP, Brunsmann F, Berger W, van Kerkhoff EP, van de Pol TJ, Wieringa B, Pawlowitzki IH, Ropers HH. 1990a. Cloning of the breakpoints of a deletion associated with choroideremia. *Hum Genet* **86:** 61–64. doi:10.1007/BF00205174

Cremers FP, Sankila EM, Brunsmann F, Jay M, Jay B, Wright A, Pinckers AJ, Schwartz M, van de Pol DJ, Wieringa B, et al. 1990b. Deletions in patients with classical choroideremia vary in size from 45 kb to several megabases. *Am J Hum Genet* **47:** 622–628.

Cremers FP, van de Pol DJ, van Kerkhoff LP, Wieringa B, Ropers HH. 1990c. Cloning of a gene that is rearranged in patients with choroideraemia. *Nature* **347:** 674–677. doi:10.1038/347674a0

Cremers FP, Armstrong SA, Seabra MC, Brown MS, Goldstein JL. 1994. REP-2, a Rab escort protein encoded by the choroideremia-like gene. *J Biol Chem* **269:** 2111–2117. doi:10.1016/S0021-9258(17)42142-9

Cunha DL, Richardson R, Tracey-White D, Abbouda A, Mitsios A, Horneffer-van der Sluis V, Takis P, Owen N, Skinner J, Welch AA, et al. 2021. REP1 deficiency causes systemic dysfunction of lipid metabolism and oxidative stress in choroideremia. *JCI Insight* **6:** e146934. doi:10.1172/jci.insight.146934

De Silva SR, Arno G, Robson AG, Fakin A, Pontikos N, Mohamed MD, Bird AC, Moore AT, Michaelides M, Webster AR, et al. 2021. The X-linked retinopathies: physiological insights, pathogenic mechanisms, phenotypic features and novel therapies. *Prog Retin Eye Res* **82:** 100898. doi:10.1016/j.preteyeres.2020.100898

Dimopoulos IS, Hoang SC, Radziwon A, Binczyk NM, Seabra MC, MacLaren RE, Somani R, Tennant MTS, MacDonald IM. 2018. Two-year results after AAV2-mediated gene therapy for choroideremia: the Alberta experience. *Am J Ophthalmol* **193:** 130–142. doi:10.1016/j.ajo.2018.06.011

Duong TT, Vasireddy V, Ramachandran P, Herrera PS, Leo L, Merkel C, Bennett J, Mills JA. 2018. Use of induced pluripotent stem cell models to probe the pathogenesis of choroideremia and to develop a potential treatment. *Stem Cell Res* **27:** 140–150. doi:10.1016/j.scr.2018.01.009

Edwards TL, Jolly JK, Groppe M, Barnard AR, Cottriall CL, Tolmachova T, Black GC, Webster AR, Lotery AJ, Holder GE, et al. 2016. Visual acuity after retinal gene therapy for choroideremia. *N Engl J Med* **374:** 1996–1998. doi:10.1056/NEJMc1509501

Fischer MD, Ochakovski GA, Beier B, Seitz IP, Vaheb Y, Kortuem C, Reichel FFL, Kuehlewein L, Kahle NA, Peters T, et al. 2019. Efficacy and safety of retinal gene therapy using adeno-associated virus vector for patients with choroideremia: a randomized clinical trial. *JAMA Ophthalmol* **137:** 1247–1254. doi:10.1001/jamaophthalmol.2019.3278

Fischer MD, Ochakovski GA, Beier B, Seitz IP, Vaheb Y, Kortuem C, Reichel FFL, Kuehlewein L, Kahle NA, Peters T, et al. 2020. Changes in retinal sensitivity after gene therapy in choroideremia. *Retina* **40:** 160–168. doi:10.1097/IAE.0000000000002360

Foote KG, Rinella N, Tang J, Bensaid N, Zhou H, Zhang Q, Wang RK, Porco TC, Roorda A, Duncan JL. 2019. Cone structure persists beyond margins of short-wavelength autofluorescence in choroideremia. *Invest Ophthalmol Vis Sci* **60:** 4931–4942. doi:10.1167/iovs.19-27979

Forsius H, Hyvärinen L, Nieminen H, Flower R. 1977. Fluorescein and indocyanine green fluorescence angiography in study of affected males and in female carriers with choroidermia. A preliminary report. *Acta Ophthalmol* **55:** 459–470. doi:10.1111/j.1755-3768.1977.tb06123.x

François J. 1971. Sex-linked chorioretinal heredodegenerations. *Birth Defects Orig Artic Ser* **7:** 99–116.

Fry LE, Patrício MI, Jolly JK, Xue K, MacLaren RE. 2021. Expression of Rab prenylation pathway genes and relation to disease progression in choroideremia. *Transl Vis Sci Technol* **10:** 12. doi:10.1167/tvst.10.8.12

Fukushima M, Inoue T, Miyai T, Obata R. 2020. Retinal dystrophy associated with Danon disease and pathogenic mechanism through LAMP2-mutated retinal pigment epithelium. *Euro J Ophthalmol* **30:** 570–578. doi:10.1177/1120672119832183

Goedbloed J. 1942. Mode of inheritance in chorioideremia. *Ophthalmologica* **104:** 308–315. doi:10.1159/000300062

Gordiyenko NV, Fariss RN, Zhi C, MacDonald IM. 2010. Silencing of the *CHM* gene alters phagocytic and secretory pathways in the retinal pigment epithelium. *Invest Ophthalmol Vis Sci* **51:** 1143–1150. doi:10.1167/iovs.09-4117

Han RC, Fry LE, Kantor A, McClements ME, Xue K, MacLaren RE. 2021. Is subretinal AAV gene replacement still

the only viable treatment option for choroideremia? *Expert Opin Orphan Drugs* **9:** 13–24. doi:10.1080/21678707.2021.1882300

Hasegawa A, Noda K, Fujiya A, Hirooka K, Anzai T, Ishida S. 2022. Outer retinal abnormalities in a patient with Danon disease. *Retinal Cases Brief Rep* **16:** 619–621. doi:10.1097/ICB.0000000000001043

Hildebrand MS, de Silva MG, Tan TY, Rose E, Nishimura C, Tolmachova T, Hulett JM, White SM, Silver J, Bahlo M, et al. 2007. Molecular characterization of a novel X-linked syndrome involving developmental delay and deafness. *Am J Med Genet A* **143A:** 2564–2575. doi:10.1002/ajmg.a.31995

Hull S, Mukherjee R, Holder GE, Moore AT, Webster AR. 2016. The clinical features of retinal disease due to a dominant mutation in RPE65. *Mol Vis* **22:** 626–635.

Hume AN, Collinson LM, Rapak A, Gomes AQ, Hopkins CR, Seabra MC. 2001. Rab27a regulates the peripheral distribution of melanosomes in melanocytes. *J Cell Biol* **152:** 795–808. doi:10.1083/jcb.152.4.795

Iossa S, Costa V, Corvino V, Auletta G, Barruffo L, Cappellani S, Ceglia C, Cennamo G, D'Adamo AP, D'Amico A, et al. 2015. Phenotypic and genetic characterization of a family carrying two Xq21.1-21.3 interstitial deletions associated with syndromic hearing loss. *Mol Cytogenet* **8:** 18. doi:10.1186/s13039-015-0120-0

Jacobson SG, Cideciyan AV, Sumaroka A, Aleman TS, Schwartz SB, Windsor EA, Roman AJ, Stone EM, MacDonald IM. 2006. Remodeling of the human retina in choroideremia: rab escort protein 1 (*REP-1*) mutations. *Invest Ophthalmol Vis Sci* **47:** 4113–4120. doi:10.1167/iovs.06-0424

Jauregui R, Park KS, Tanaka AJ, Cho A, Paavo M, Zernant J, Francis JH, Allikmets R, Sparrow JR, Tsang SH. 2019. Spectrum of disease severity and phenotype in choroideremia carriers. *Am J Ophthalmol* **207:** 77–86. doi:10.1016/j.ajo.2019.06.002

Jauregui R, Cho A, Oh JK, Tanaka AJ, Sparrow JR, Tsang SH. 2020. Phenotypic expansion of autosomal dominant retinitis pigmentosa associated with the D477G mutation in *RPE65*. *Cold Spring Harb Mol Case Stud* **6:** a004952. doi:10.1101/mcs.a004952

Jolly JK, Xue K, Edwards TL, Groppe M, MacLaren RE. 2017. Characterizing the natural history of visual function in choroideremia using microperimetry and multimodal retinal imaging. *Invest Ophthalmol Vis Sci* **58:** 5575–5583. doi:10.1167/iovs.17-22486

Kärnä J. 1986. Choroideremia. A clinical and genetic study of 84 Finnish patients and 126 female carriers. *Acta Ophthalmol Suppl* **176:** 1–68.

Kiang AS, Kenna PF, Humphries MM, Ozaki E, Koenekoop RK, Campbell M, Farrar GJ, Humphries P. 2020. Properties and therapeutic implications of an enigmatic D477G RPE65 variant associated with autosomal dominant retinitis pigmentosa. *Genes (Basel)* **11:** 1420. doi:10.3390/genes11121420

Kmoch S, Majewski J, Ramamurthy V, Cao S, Fahiminiya S, Ren H, MacDonald IM, Lopez I, Sun V, Keser V, et al. 2015. Mutations in PNPLA6 are linked to photoreceptor degeneration and various forms of childhood blindness. *Nat Commun* **6:** 5614. doi:10.1038/ncomms6614

Kwon W, Freeman SA. 2020. Phagocytosis by the retinal pigment epithelium: recognition, resolution, recycling. *Front Immunol* **11:** 604205. doi:10.3389/fimmu.2020.604205

Kyhn MV, Kiilgaard JF, Lopez AG, Scherfig E, Prause JU, la Cour M. 2008. Functional implications of short-term retinal detachment in porcine eyes: study by multifocal electroretinography. *Acta Ophthalmol* **86:** 18–25. doi:10.1111/j.1600-0420.2007.00983.x

Lam BL, Davis JL, Gregori NZ, MacLaren RE, Girach A, Verriotto JD, Rodriguez B, Rosa PR, Zhang X, Feuer WJ. 2019. Choroideremia gene therapy phase 2 clinical trial: 24-month results. *Am J Ophthalmol* **197:** 65–73. doi:10.1016/j.ajo.2018.09.012

Larijani B, Hume AN, Tarafder AK, Seabra MC. 2003. Multiple factors contribute to inefficient prenylation of Rab27a in Rab prenylation diseases. *J Biol Chem* **278:** 46798–46804. doi:10.1074/jbc.M307799200

Lewis RA, Nussbaum RL, Ferrell R. 1985. Mapping X-linked ophthalmic diseases. Provisional assignment of the locus for choroideremia to Xq13-q24. *Ophthalmology* **92:** 800–806. doi:10.1016/S0161-6420(85)33956-8

Liang S, Jiang N, Li S, Jiang X, Yu D. 2017. A maternally inherited 8.05 Mb Xq21 deletion associated with choroideremia, deafness, and mental retardation syndrome in a male patient. *Mol Cytogenet* **10:** 23. doi:10.1186/s13039-017-0324-6

Lyon MF. 1961. Gene action in the X-chromosome of the mouse (*Mus musculus* L). *Nature* **190:** 372–373. doi:10.1038/190372a0

Ma KK, Lin J, Boudreault K, Chen RW, Tsang SH. 2017. Phenotyping choroideremia and its carrier state with multimodal imaging techniques. *Retin Cases Brief Rep* **11**(Suppl 1)**:** S178–S181.

MacLaren RE, Groppe M, Barnard AR, Cottriall CL, Tolmachova T, Seymour L, Clark KR, During MJ, Cremers FP, Black GC, et al. 2014. Retinal gene therapy in patients with choroideremia: initial findings from a phase 1/2 clinical trial. *Lancet* **383:** 1129–1137. doi:10.1016/S0140-6736(13)62117-0

Mauthner L. 1872. Ein fall von choroideremie. *Ber Naturwissensch-med Ver Inssbruck* **2:** 191–197.

Merry DE, Lesko JG, Sosnoski DM, Lewis RA, Lubinsky M, Trask B, van den Engh G, Collins FS, Nussbaum RL. 1989. Choroideremia and deafness with stapes fixation: a contiguous gene deletion syndrome in Xq21. *Am J Hum Genet* **45:** 530–540.

Moosajee M, Tulloch M, Baron RA, Gregory-Evans CY, Pereira-Leal JB, Seabra MC. 2009. Single *choroideremia* gene in nonmammalian vertebrates explains early embryonic lethality of the zebrafish model of choroideremia. *Invest Ophthalmol Vis Sci* **50:** 3009–3016. doi:10.1167/iovs.08-2755

Muniz A, Villazana-Espinoza ET, Hatch AL, Trevino SG, Allen DM, Tsin AT. 2007. A novel cone visual cycle in the cone-dominated retina. *Exp Eye Res* **85:** 175–184. doi:10.1016/j.exer.2007.05.003

Murro V, Mucciolo DP, Giorgio D, Sodi A, Passerini I, Virgili G, Rizzo S. 2020. Optical coherence tomography angiography (OCT-A) in choroideremia (CHM) carriers. *Ophthalmic Genet* **41:** 146–151. doi:10.1080/13816810.2020.1747086

Cold Spring Harbor Perspectives in Medicine
www.perspectivesinmedicine.org

Cold Spring Harbor Perspectives in Medicine
www.perspectivesinmedicine.org

Nussbaum RL, Lewis RA, Lesko JG, Ferrell R. 1985. Choroideremia is linked to the restriction fragment length polymorphism DXYS1 at XQ13-21. *Am J Hum Genet* **37:** 473–481.

Nussbaum RL, Lesko JG, Lewis RA, Ledbetter SA, Ledbetter DH. 1987. Isolation of anonymous DNA sequences from within a submicroscopic X chromosomal deletion in a patient with choroideremia, deafness, and mental retardation. *Proc Natl Acad Sci* **84:** 6521–6525. doi:10.1073/pnas.84.18.6521

O'Neil E, Serrano L, Scoles D, Cunningham KE, Han G, Chiang J, Bennett J, Aleman TS. 2019. Detailed retinal phenotype of Boucher–Neuhäuser syndrome associated with mutations in *PNPLA6* mimicking choroideremia. *Ophthalmic Genet* **40:** 267–275. doi:10.1080/13816810.2019.1605392

Ørstavik KH. 2006. Skewed X inactivation in healthy individuals and in different diseases. *Acta Paediatr Suppl* **95:** 24–29. doi:10.1080/08035320600618783

Ortiz-Ramirez GY, Villanueva-Mendoza C, Zenteno Ruiz JC, Reyes M, Cortés-González V. 2020. Autofluorescence in female carriers with choroideremia: a familial case with a novel mutation in the *CHM* gene. *Ophthalmic Genet* **41:** 625–628. doi:10.1080/13816810.2020.1810283

Paavo M, Carvalho JRL Jr, Lee W, Sengillo JD, Tsang SH, Sparrow JR. 2019. Patterns and intensities of near-infrared and short-wavelength fundus autofluorescence in choroideremia probands and carriers. *Invest Ophthalmol Vis Sci* **60:** 3752–3761. doi:10.1167/iovs.19-27366

Patrício MI, Barnard AR, Cox CI, Blue C, MacLaren RE. 2018. The biological activity of AAV vectors for choroideremia gene therapy can be measured by in vitro prenylation of RAB6A. *Mol Ther Methods Clin Dev* **9:** 288–295. doi:10.1016/j.omtm.2018.03.009

Perez-Cano HJ, Garnica-Hayashi RE, Zenteno JC. 2009. *CHM* gene molecular analysis and X-chromosome inactivation pattern determination in two families with choroideremia. *Am J Med Genet A Part A* **149a:** 2134–2140. doi:10.1002/ajmg.a.32727

Pfau M, Jolly JK, Wu Z, Denniss J, Lad EM, Guymer RH, Fleckenstein M, Holz FG, Schmitz-Valckenberg S. 2021. Fundus-controlled perimetry (microperimetry): application as outcome measure in clinical trials. *Prog Retin Eye Res* **82:** 100907. doi:10.1016/j.preteyeres.2020.100907

Poloschek CM, Kloeckener-Gruissem B, Hansen LL, Bach M, Berger W. 2008. Syndromic choroideremia: sublocalization of phenotypes associated with Martin–Probst deafness mental retardation syndrome. *Invest Ophthalmol Vis Sci* **49:** 4096–4104. doi:10.1167/iovs.08-2044

Sankila EM, Bruns GA, Schwartz M, Nikoskelainen E, Niebuhr E, Hodgson SV, Wright AF, de la Chapelle A. 1990. DXS26 (HU16) is located in Xq21.1. *Hum Genet* **85:** 117–120. doi:10.1007/BF00276335

Sankila EM, Tolvanen R, van den Hurk JA, Cremers FP, de la Chapelle A. 1992. Aberrant splicing of the CHM gene is a significant cause of choroideremia. *Nat Genet* **1:** 109–113. doi:10.1038/ng0592-109

Schwartz M, Rosenberg T, Niebuhr E, Lundsteen C, Sardemann H, Andersen O, Yang HM, Lamm LU. 1986. Choroideremia: further evidence for assignment of the locus to Xq13-Xq21. *Hum Genet* **74:** 449–452. doi:10.1007/BF00280505

Seabra MC. 1996. New insights into the pathogenesis of choroideremia: a tale of two REPs. *Ophthalmic Genet* **17:** 43–46. doi:10.3109/13816819609057869

Seabra MC, Ho YK, Anant JS. 1995. Deficient geranylgeranylation of Ram/Rab27 in choroideremia. *J Biol Chem* **270:** 24420–24427. doi:10.1074/jbc.270.41.24420

Secondi R, Kong J, Blonska AM, Staurenghi G, Sparrow JR. 2012. Fundus autofluorescence findings in a mouse model of retinal detachment. *Invest Ophthalmol Vis Sci* **53:** 5190–5197. doi:10.1167/iovs.12-9672

Seitz IP, Jolly JK, Dominik Fischer M, Simunovic MP. 2018. Colour discrimination ellipses in choroideremia. *Graefes Arch Clin Exp Ophthalmol* **256:** 665–673. doi:10.1007/s00417-018-3921-0

Sieving PA, Niffenegger JH, Berson EL. 1986. Electroretinographic findings in selected pedigrees with choroideremia. *Am J Ophthalmol* **101:** 361–367. doi:10.1016/0002-9394(86)90832-9

Simunovic MP, Jolly JK, Xue K, Edwards TL, Groppe M, Downes SM, MacLaren RE. 2016. The spectrum of CHM gene mutations in choroideremia and their relationship to clinical phenotype. *Invest Ophthalmol Vis Sci* **57:** 6033–6039. doi:10.1167/iovs.16-20230

Stevanovic M, Cehajic Kapetanovic J, Jolly JK, MacLaren RE. 2020. A distinct retinal pigment epithelial cell autofluorescence pattern in choroideremia predicts early involvement of overlying photoreceptors. *Acta Ophthalmol* **98:** e322–e327. doi:10.1111/aos.14281

Taylor MRG, Adler ED. 1993. Danon disease. In *Genereviews* (ed. Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Gripp KW, Mirzaa GM, Amemiya A). University of Washington, Seattle.

Taylor LJ, Josan AS, Pfau M, Simunovic MP, Jolly JK. 2022. Scotopic microperimetry: evolution, applications and future directions. *Clin Exp Optom* **105:** 793–800. doi:10.1080/08164622.2021.2023477

Thompson DA, Constable PA, Liasis A, Walters B, Esteban MT. 2016. The physiology of the retinal pigment epithelium in Danon disease. *Retina* **36:** 629–638. doi:10.1097/IAE.0000000000000736

Tolmachova T, Anders R, Abrink M, Bugeon L, Dallman MJ, Futter CE, Ramalho JS, Tonagel F, Tanimoto N, Seeliger MW, et al. 2006. Independent degeneration of photoreceptors and retinal pigment epithelium in conditional knockout mouse models of choroideremia. *J Clin Invest* **116:** 386–394. doi:10.1172/JCI26617

Tolmachova T, Tolmachov OE, Barnard AR, de Silva SR, Lipinski DM, Walker NJ, Maclaren RE, Seabra MC. 2013. Functional expression of Rab escort protein 1 following AAV2-mediated gene delivery in the retina of choroideremia mice and human cells ex vivo. *J Mol Med* **91:** 825–837. doi:10.1007/s00109-013-1006-4

Vaché C, Torriano S, Faugère V, Erkilic N, Baux D, Garcia-Garcia G, Hamel CP, Meunier I, Zanlonghi X, Koenig M, et al. 2019. Pathogenicity of novel atypical variants leading to choroideremia as determined by functional analyses. *Hum Mutat* **40:** 31–35. doi:10.1002/humu.23671

Wavre-Shapton ST, Tolmachova T, Lopes da Silva M, Futter CE, Seabra MC. 2013. Conditional ablation of the choroideremia gene causes age-related changes in mouse retinal

pigment epithelium. *PLoS ONE* **8:** e57769. doi:10.1371/journal.pone.0057769

Wu AL, Wang JP, Tseng YJ, Liu L, Kang YC, Chen KJ, Chao AN, Yeh LK, Chen TL, Hwang YS, et al. 2018. Multimodal imaging of mosaic retinopathy in carriers of hereditary x-linked recessive diseases. *Retina* **38:** 1047–1057. doi:10.1097/IAE.0000000000001629

Wu S, Sun Z, Zhu T, Weleber RG, Yang P, Wei X, Pennesi ME, Sui R. 2021. Novel variants in PNPLA6 causing syndromic retinal dystrophy. *Exp Eye Res* **202:** 108327. doi:10.1016/j.exer.2020.108327

Xue K, Oldani M, Jolly JK, Edwards TL, Groppe M, Downes SM, MacLaren RE. 2016. Correlation of optical coherence tomography and autofluorescence in the outer retina and choroid of patients with choroideremia. *Invest Ophthalmol Vis Sci* **57:** 3674–3684. doi:10.1167/iovs.15-18364

Xue K, Jolly JK, Barnard AR, Rudenko A, Salvetti AP, Patrício MI, Edwards TL, Groppe M, Orlans HO, Tolmachova T, et al. 2018. Beneficial effects on vision in patients undergoing retinal gene therapy for choroideremia. *Nat Med* **24:** 1507–1512. doi:10.1038/s41591-018-0185-5

Cold Spring Harbor Perspectives in Medicine
www.perspectivesinmedicine.org

Choroideremia: Toward Regulatory Approval of Retinal Gene Therapy

Imran H. Yusuf and Robert E. MacLaren

Cold Spring Harb Perspect Med 2023; doi: 10.1101/cshperspect.a041279 originally published online June 5, 2023

Subject Collection Retinal Disorders: Genetic Approaches to Diagnosis and Treatment

For additional articles in this collection, see http://perspectivesinmedicine.cshlp.org/cgi/collection/