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Pathogenic mechanisms and the prospect of gene therapy for choroideremia

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Abstract

Introduction—Choroideremia is a rare, X-linked disorder recognized by its specific ocular phenotype as a progressive degenerative retinopathy resulting in blindness. New therapeutic approaches, primarily based on genetic mechanisms, have emerged that aim to prevent the progressive vision loss.

Areas covered—This article will review the research that has progressed incrementally over the past two decades from mapping to gene discovery, uncovering the presumed mechanisms triggering the retinopathy to preclinical testing of potential therapies.

Expert opinion—While still in an evaluative phase, the introduction of gene replacement as a potential therapy has been greeted with great enthusiasm by patients, advocacy groups and the medical community.

Keywords

choroideremia; gene therapy; REPI; retina

1. Introduction

1.1 Defining the disorder clinically

Choroideremia (CHM) is an X-linked, progressive retinal degenerative disease with an estimated prevalence of 1 in 50,000 – 1 in 100,000 individuals [1,2]. Affected male patients

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Declaration of interest

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experience decreased night vision in early childhood, followed by reduced peripheral vision and eventually complete blindness in most cases by the sixth decade of life.

The progressive degeneration in CHM affects three structural layers of the eye: neuroretina, retinal pigment epithelium (RPE) and choroid. Signs of atrophy in these layers and a reduction in retinal sensitivity are clinically evident well before affected patients report significant vision loss [3]. There is still uncertainty surrounding the general order of cell death in CHM; that is, whether the neuroretina, RPE and choroid are all primarily affected, or whether one or more of these layers is secondarily affected [4]. Psychophysical tests, however, have revealed reductions in retinal sensitivity before the onset of significant degeneration, implying impairment of the visual cycle to some extent [3]. It is perhaps not surprising that deficiency of the CHM gene product leads to a functional impairment before RPE cell death, but it is not clear whether this is related specifically to deficiency or if it is a nonspecific consequence of general stress to the RPE cells.

The clinical diagnosis of CHM usually includes the following: fundus examination; visual field (VF); electroretinography (ERG); fundus autofluorescence (FAF); and an evaluation of family history. Fundoscopic examination in affected male patients shows patches of chorioretinal atrophy and RPE degeneration in the mid-periphery (Figure 1A and B). This anatomical degeneration usually corresponds to peripheral VF deficits. The full-field ERG shows abnormal scotopic responses, which correlate symptomatically with reduced night vision, typically the first reported symptom in affected patients. FAF, which is used to see the natural autofluorescence of lipofuscin in RPE cells, is recorded *in vivo* by using a confocal scanning laser ophthalmoscope [5] or fundus photography [6]. Regions of RPE atrophy are detected as areas of reduced autofluorescence. Regions of increased autofluorescence are believed to be associated with increased lipofuscin accumulation in RPE cells [5]. In CHM patients, there is a loss of autofluorescence in areas corresponding to regions of chorioretinal atrophy, and these regions are surrounded by areas of remaining autofluorescence that demarcate residual RPE tissue (Figure 1D and E). Finally, a family history of X-linked disease helps to further support the diagnosis.

Carrier females generally present with subnormal ERGs [7], and they have a characteristic pattern of diffuse mottled hyperpigmentation upon fundus examination and a speckled pattern of reduced and increased autofluorescence upon FAF (Figure 1C and F) [8]. Carrier females are generally asymptomatic in early years, but some experience night blindness and reduced peripheral vision in late adulthood. The findings in the carrier can also be seen as a progressive disease [9,10].

1.2 History of gene discovery

In 1985, linkage analysis studies first mapped the gene responsible for CHM to a region spanning Xq13-q21 [11]. A study published 1 year later corroborated these initial findings [12]. In 1989, an elegant study by Merry *et al.*, involving patients with a contiguous gene deletion syndrome that included CHM and deafness, helped narrow the locus of the causative gene to Xq21 [13]. Follow-up studies, including an analysis of a large population of CHM Finnish families [14], provided additional insights and helped narrow the search for the *CHM* gene even further [15]. In 1990, Cremers *et al.* reported that the *CHM* gene had

been successfully cloned [16]. Shortly thereafter, reports were published that demonstrated a functional relationship between the *CHM* gene product and Rab geranylgeranyl transferase (RabGGT) [17,18]. A 1993 report coined the term “Rab escort protein” (REP) for the protein product of the *CHM* gene based on its proposed function [19].

CHM is caused by mutations in the *CHM* gene, which encodes Rab escort protein 1 (REP1). It is generally accepted that most mutations in *CHM* – from single-nucleotide mutations to entire gene deletions – are loss-of-function mutations that lead to absent or truncated REP1 protein [20]. In addition, two missense mutations have been reported to date [21,22]. *In vitro* functional analysis of one of the missense variants (p.H507R) demonstrated the inability of the inactive REP1 to bind to the RabGGT [22]. A list of all CHM causative mutations can be found in the Leiden Open Variation Database at www.lovd.nl.

1.3 Syndromic cases

CHM male patients and female carriers experience disease consequences that are restricted to the eye. However, given the fact that up to 15% of mutations in the *CHM* gene are entire or partial gene deletions [20], it is not surprising that contiguous gene deletions on the X chromosome that include deletion of Xq21 may rarely cause syndromic cases of CHM. Reported cases have included patients with comorbidities such as mental and motor retardation, sensorineural deafness, cleft lip and palate, and other clinical phenotypes [23–27]. A 2011 study reported on a patient who presented with CHM, as well as dysplasia of the auricular system, patent ductus arteriosus and enamel hypoplasia [28]. Another study reported CHM associated with X-linked distal spinal muscular atrophy and Martin-Probst deafness mental retardation syndrome [15].

Interestingly, a small number of studies have reported syndromic cases that involved genomic translocations affecting the X chromosome and not contiguous gene deletions. One such case involved an X-autosome chromosomal translocation that resulted in CHM and ectodermal dysplasia [29]. Another case also involved an X-autosome translocation; the patient had CHM, mild sensorineural deafness and primary amenorrhea [30]. Although representing a minor fraction of patients with CHM, it is important to differentiate these cases from the majority of nonsyndromic CHM for accurate diagnosis, as well as for appropriate targeting in future gene replacement therapies.

1.4 Molecular and genetic diagnosis

While affected male CHM patients generally have characteristic clinical findings, as described above, molecular diagnosis is always required to corroborate clinical findings. Indeed, molecular and genetic analyses can sometimes point to a different diagnosis, as has been reported previously [31]. Such confirmatory analyses will be necessary for patients who will be enrolled in upcoming clinical trials.

To date, CHM has only been reported to be caused by mutations involving the *CHM* gene. The first step in corroborating the clinical phenotype of CHM is sequence analysis of the *CHM* gene [2,20,32]. If this approach fails to identify a mutation, further analyses are undertaken to look for deletions, duplications or genomic translocations. Protein testing may

also be performed. Most mutations in – and deletions of – *CHM* lead to absent or severely truncated REP1 protein, which can be detected by immunoblot analysis of peripheral leukocytes from affected male patients [33]. Given that essentially all mutations in *CHM* lead to absent or severely truncated REP1, analysis of peripheral blood for REP1 protein expression has been used to help confirm CHM as a diagnosis and differentiate it from other diseases [33]. The normally ubiquitous expression of REP1 allows for such a test to have diagnostic value. However, this type of analysis is only available on a limited, research basis.

1.5 Pathogenesis

1.5.1 Trafficking—There are two isoforms of REP: REP1 and REP2. They participate in key cellular functions, including intracellular trafficking and secretion by recognizing Rab proteins [34]. For example, certain Rabs are involved in vesicular transport of rhodopsin from the Golgi apparatus to the connecting cilium in photoreceptor cells [35]. REPs recognize Rabs in the cytosol and serve as their molecular chaperones, bringing them to RabGGTs, which modify Rabs via addition of geranylgeranyl groups; this process is known as prenylation, and it is essential for Rabs to be able to attach to organelle lipid bilayers [36]. In CHM, normal REP1 is absent; however, it appears that REP2 activity can compensate for this deficiency in most tissues, except for the RPE and possibly the photoreceptors and choroid.

Human Rab27A – as well as its rat homologue, which has been shown to be expressed in the RPE and choriocapillaris – accumulates in the unprenylated form in the cytosol in cells of CHM patients [37–39]. This was initially assumed to be due to preferential REP1 prenylation of this Rab protein. Recent evidence [40–42] suggests that although Rab27A can be prenylated by REP-2, this reaction is inhibited in CHM by competing Rabs that have higher binding affinity for the REP prenylation complex. Loss of REP1 function in CHM will therefore primarily reduce prenylation efficiency of Rab27A and other low-affinity Rabs (Rab27B, Rab38 and Rab42) [40]. The exact mechanisms by which unprenylated Rabs contribute to cell death and progressive chorioretinal atrophy in CHM patients are still unknown.

Some experimental evidence suggests that the severity of the CHM phenotype correlates with the severity of defects in processes of intracellular trafficking [43]. These processes include pathways of phagocytosis, intracellular trafficking and secretion, such as vesicular transport and neurotransmission [43]. Could a reduction in the pool of isoprenoids with pharmacologic agents that affect REP1-mediated prenylation of Rabs have an effect on the CHM phenotype? Hydroxy-3-methyl-glutaryl-CoA reductase inhibitors, known as statins, have been reported to deplete geranylgeranylpyrophosphate and suppress small GTPases, particularly Rabs [44]. An Internet-based survey of patients was undertaken to determine if statins had any effect on visual function in males with CHM. Whereas patients taking statins had poorer visual function, these patients were also older and more likely to be on statins [45]. Further studies involving *in vitro* human cellular models as well as animal models of CHM are required to elucidate a possible deleterious effect of statin use in CHM.

1.5.2 X-inactivation—X-inactivation, also known as lyonization, is the process by which one of the two X chromosomes present in cells of females is randomly inactivated, leaving only one “active” X chromosome per cell. The ocular manifestations of several ocular diseases are influenced by X-inactivation, including X-linked retinitis pigmentosa (XLRP), X-linked albinism, and CHM [46].

Some CHM female carriers have been known to have a more severe disease phenotype than what is typical; such phenotypes have been attributed to skewed X-inactivation in which the “normal” *CHM* allele is preferentially inactivated, although some studies have challenged this conclusion [47]. Carrel and Willard [48] showed that the *CHM* gene escapes inactivation with reduced but detectable expression from inactivated X chromosomes in all cells. Other studies have shown a mosaic pattern of cone cell function in female CHM carriers, suggesting a relationship between X-inactivation and phenotype [49].

1.6 Differential diagnosis: retinitis pigmentosa and gyrate atrophy

As has been previously reported [31], the clinical presentation of CHM can have features that overlap with other retinal dystrophies. Differential diagnoses primarily include retinitis pigmentosa (RP) and gyrate atrophy.

RP is a progressive blinding disease that has several features that overlap with CHM; this is particularly true for XLRP. In both conditions, affected patients are generally males who present with reduced night vision in early adulthood, followed by peripheral VF deficits. Moreover, both conditions are X-linked and thus show similar patterns of inheritance. In XLRP, central vision is generally lost during the third or fourth decade of life, compared to the fifth or sixth decade in CHM. Fundus examination further helps to differentiate XLRP from CHM. First, XLRP patients typically present with extensive pigment migration; this does not happen in CHM patients. Second, severe chorioretinal atrophy, which exposes the underlying sclera, occurs in CHM but not RP. This can be visualized using FAF. In CHM, the optic nerve remains relatively normal until late in the disease course, whereas in XLRP the optic nerve head can appear to have a waxy pallor at an early age. Finally, in RP, narrowing of retinal blood vessels is typically seen; this does not occur in CHM. Female carriers of CHM and XLRP also have some notable differences; the former present with patches of chorioretinal degeneration, whereas the latter often present with the “bone spicules” that are characteristic of the disease in affected males. Female CHM and RP carriers have different FAF patterns; the latter have a radial FAF pattern [42], whereas the former have a speckled pattern of areas with reduced and increased autofluorescence [20]. This most likely reflects the different patterns of growth of the RPE and photoreceptors, respectively. The radial FAF pattern seen in XLRP most likely represents the late enlargement of the fovea in the early postnatal stages in which clones containing either normal or mutant X chromosomes would be stretched out on a radial axis. This does not occur with the RPE, which differentiates relatively uniformly.

Gyrate atrophy was previously thought to be a subtype of CHM given that its clinical presentation is similar and includes progressive degeneration of the RPE and choroid; patient symptoms are also similar, with early-onset night blindness followed by progressive loss of peripheral vision and visual acuity (VA). The underlying causes of gyrate atrophy are

mutations in the ornithine aminotransferase (*OAT*) gene, which leads to toxic levels of plasma ornithine that damage RPE and choroid. Gyrate atrophy can be clinically distinguished from CHM by systemic signs of a proximal myopathy and the generalized hyperpigmentation of remaining RPE in gyrate atrophy. In addition, early cataract formation is a feature of gyrate atrophy not generally seen in CHM [43]. Analysis of serum or plasma ornithine levels can also be used to differentiate the two diseases, as can analysis of the *OAT* gene. Moreover, a family history of X-linked inheritance is seen in CHM but not gyrate atrophy, which is inherited in an autosomal recessive manner.

Diffuse choroidal sclerosis with autosomal dominant inheritance can mimic CHM [50]. This disorder is characterized by progressive thinning and loss of choriocapillaris beginning in midlife and resulting in severe loss of vision by later years [50]. Recessive mutations in *RPE65* may also present with RPE defects in later stages and thus give a similar appearance to CHM. It is important to note that the onset of visual dysfunction in these patients is at a much younger age compared to CHM, while RPE defects occur at a later stage.

2. Current management strategies

There are currently no treatment options available for CHM patients; however, there are several approaches to managing the disease and its progression as discussed below.

2.1 Cataract surgery

Although a common feature in rod-cone diseases, such as RP, the prevalence of cataract does not seem to be higher in CHM than the general population, and it may even be slightly lower [51]. It is unlikely that disease mechanisms in CHM contribute to cataract formation [45]. As in the general population, there is a correlation between increased age and incidence of cataract in CHM patients [51]; those with posterior subcapsular cataract undergo cataract surgery, as needed. Cataract surgery in CHM patients does not seem to add any additional risk of intra- or early postoperative complications according to a recent case series, which is an important consideration in gene therapy as vitrectomy surgery is known to induce cataract formation [52].

2.2 Anti-vascular endothelial growth factor therapy for choroidal neovascularization

Anti-vascular endothelial growth factor (VEGF) therapy has been used to treat choroidal neovascularization (CNV) associated with various ocular diseases, most notably neovascular age-related macular degeneration (AMD). While the majority of CHM patients maintain normal VA until adulthood, there have been reports of cases in which the patients presented with CNV. An example of peripapillary CNV development in CHM can be seen in Figure 1B (white arrowhead). The first case of CNV in CHM reported in the literature involved a young boy, who was 14 years old [53]. In 2014, the first trial of intravitreal bevacizumab, a potent anti-VEGF drug, was attempted [54]. The authors of the study reported that the patient, a 13-year-old boy, experienced mild improvement in VA following treatment. Another report of a 30-year-old male CHM patient who presented with CNV in one eye and was not treated found that the CNV regressed at 6 months of follow-up; this correlated with significant improvements in his VA [55]. Spontaneous resolution of CNV, and subsequent

formation of subretinal fibrotic scarring, can occur in CHM patients [56]. Given the unknown prevalence of CNV in CHM patients, and the relative sparse evidence for the efficacy of anti-VEGF therapies to treat CHM-associated CNV, their use is not routinely recommended at this point.

2.3 Cystoid maculopathy

Macular edema, which involves accumulation of fluid in the macular region, has been known to occur in a variety of ophthalmic conditions, including RP and diabetic retinopathy. Cystoid macular edema (CME) – which gets its name from its cystic-like appearance – has only more recently been explored in CHM. A 2011 study reported the prevalence of CME in 16 CHM patients using spectral domain optical coherence tomography (SD-OCT) and found some degree of CME in 62.5% of patients [57]. The same group later reported treatment of CME in two CHM patients using topical dorzolamide, a form of carbonic anhydrase inhibitor [58]. While this study showed an improvement in retinal thickness in the two treated patients, changes in VA and retinal sensitivity were not clinically significant. As CME can often resolve over time without treatment, there is currently no consensus on whether or not treatment of CME in CHM patients is necessary, or whether such treatment has long-term benefits for patients. This is true for CHM patients and for patients with other forms of hereditary retinal dystrophies [59]. Whether or not other treatment approaches, such as intravitreal injections of triamcinolone acetonide or laser photocoagulation – which may be effective in treating CME in some retinal dystrophies [59] – could benefit CHM patients with CME is currently unknown.

2.4 Nutritional therapies

Direct evidence for the therapeutic value of nutritional sources for CHM patients does not exist. Nevertheless, it is generally recommended that patients follow a diet rich in fresh fruit, leafy green vegetables, and foods containing omega-3 fatty acids such as docosahexaenoic acid (DHA). When such dietary items are not available, vitamin supplementation is recommended.

These recommendations are made largely on the basis of what has been found in other retinal dystrophies. Previous studies have shown that diets rich in antioxidants, DHA, and the carotenoids lutein and zeaxanthin may slow disease progression in other forms of retinal dystrophy [60]. Despite this, recent evidence has shown that some of the dietary recommendations previously made do not slow progression of retinal degeneration in some diseases [61]. The topic of antioxidants and vitamins in ocular diseases is reviewed by Grover and Samson, 2014 [60].

Two previous studies that measured macular pigment levels in CHM patients and age-matched controls found no significant difference between the two groups [62,63]; supplementation with lutein did not show any benefits in the short term with regard to VA [62], although possible long-term benefits have not been assessed. Thus, there is currently a lack of evidence to support the idea that certain dietary elements can slow the progression of CHM. Nevertheless, dietary recommendations are still made based on the fact that they are

generally thought to be safe, and their effectiveness cannot be ruled out without further studies.

3. Natural history and selection of outcome measures

3.1 Natural history

Having a strong understanding of the natural history of CHM, as in most other diseases, provides critical insights that can impact the direction of research and therapeutic strategies. Moreover, this allows objective evaluation and quantification of the benefits of clinical interventions. Without natural history data, it becomes very difficult to evaluate the efficacy of therapeutic interventions in CHM, in part because this is a slowly progressing disease.

There is now general support for the notion that there is no genotype-phenotype correlation in CHM patients. This means that the inclusion of CHM patients in clinical trials may not require distinction between CHM mutations.

Recent studies [64,65] have shown that VA begins to decline in CHM patients at approximately age 50, and the rate of this decline tends to be quite variable from one patient to the next. Data exist that demonstrate that there is less variability in VF loss than VA loss, suggesting that VF assessment may be a useful measure to evaluate treatment outcomes. The slowly progressive nature of CHM means that there is a large therapeutic window, but to document a positive treatment effect at halting degeneration, mid- to long-term follow-up will be needed.

Some groups have attempted to gain insight into the natural history of CHM using animal models. However, these models do not accurately reflect the human condition. In the CHM zebrafish model, the lack of rep1 leads to premature death in the fish; this is believed to be caused by an absence of rep2 that is seen in mammalian species [66] – the protein product of *chml* – which, in humans [2], is believed to compensate for absent or nonfunctional REP1. In mice, which do have a *Chml* gene and thus produce Rep2 protein, knockout of *Chm* is embryonic lethal in males, although carrier females present with progressive disease similar to what is seen in human male patients. The reason for species-specific differences is not known.

While animal models of CHM do not accurately recapitulate the human condition, some convincing data have been obtained from their use. Using a conditional *Chm* knockout allele in a mouse model, which allows for knockout of *Chm* in specific cell types, it has been shown that the effect of *Chm* ablation alters the prenylation of different Rab proteins in photoreceptors and RPE cells, and this selective ablation causes degeneration of the target layer [67,68]. Although these layers were shown to degenerate independently, the degeneration of the RPE accelerated that of the photoreceptors [68]. In zebrafish rep1 mutants, photoreceptor degeneration has been suggested to be nonautonomous and only dependent on contact with mutant RPE [69]. In support of that finding, depletion of REP1 in human RPE cells does not affect the internalization of photoreceptor outer segments but rather reduces their degradation within the RPE [70]. These data suggest that the primary layer affected in CHM may be the RPE, if the data from mouse and zebrafish studies can be

extrapolated to humans. This has important clinical implications, as therapeutic approaches to treat CHM may need to primarily target the RPE and secondarily the photoreceptor layer.

There is some evidence from human postmortem analyses [71] that suggests that REP1 deficiency in cone cells may not lead to cone cell death. It is rather believed that cone cell death is secondary to rod cell death, as is the case in many genetic retinal diseases that affect rod cells. Loss of rod-mediated night vision as a presenting symptom of CHM would seem to support the idea that rods die before cones, and histological evidence from a female carrier has demonstrated that REP1-deficient cone cells can survive when surrounded by REP1-expressing rod cells [71]. However, immunolabeling studies [72] have shown REP-1 expression in both rod and cone photoreceptors in a variety of mammalian species, including human and nonhuman primates. Figure 2 shows immunolocalization of REP-1 in the primate retina. The strongest immunolabeling can be observed in the outer plexiform layer (Figure 2A). Preferential labeling of cone photoreceptors and, in particular, cone pedicles is apparent upon higher magnification (Figure 2B). This, of course, begs the question: Is it important to transduce both rods and cones in gene therapy trials involving human CHM patients? Although cones may be less sensitive to REP1 deficiency than rods, it cannot be ruled out that they are nonetheless autonomously affected. Adaptive optics imaging in CHM has demonstrated that even when patients have centrally intact retinal areas with normal cone density, cone sensitivity is reduced [4]. It would therefore be unreasonable not to transduce cones in a patient's retina during a gene therapy, only to have these cells degenerate at later stages.

The question of whether or not the choroid is primarily affected has not been resolved in CHM; however, postmortem [71] and imaging studies [73] provide some support for the theory that the choroid degenerates secondary to RPE cell loss. Another study has suggested that the choriocapillaris, RPE and photoreceptors may degenerate independently [74]. The observations that CNV can develop in CHM patients, however, lend weight to the notion that the choroid, like other vascular tissues, can grow in the absence of REP1, perhaps to a limited extent when surrounded by atrophic RPE.

3.2 Outcome measures

The selection of outcome measures for interventional studies is disease-specific. For example, in clinical trials of Leber congenital amaurosis (LCA), mobility course navigation (ClinicalTrials.gov identifier NCT00999609) is a primary outcome measure. In CHM, mobility course navigation would not be a useful outcome measure given that dramatic improvements in visual function are not expected; rather, the goal is to preserve existing visual function.

Natural history information can provide valuable insight into the outcome measures that can and should be used when evaluating the efficacy and safety of any therapy in CHM patients. In general, patients included in any clinical trial will be evaluated at baseline and post-treatment – often at several time points – using objective measures and subjective information. This is true for both treatment efficacy and the occurrence of adverse events.

Objective measures may be made using any combination of the diagnostic tools that are available, including adaptive optics scanning laser ophthalmoscopy [4,73], SD-OCT [73,75], FAF [73], VA [76], VF [73], microperimetry [77], ERG [73] and others.

SD-OCT allows monitoring of thickness changes in individual retinal layers and identifying abnormalities in retinal structure [73,75]. In addition, OCT provides a useful tool to assess the outcome and safety of surgical interventions in clinical trials (for example, subfoveal gene delivery). FAF is pivotal in evaluating changes in the rate of RPE degeneration while static or kinetic perimetry is used to monitor progression of VF loss. For advanced CHM patients, defined as those with VF <15 degrees in diameter, fundus perimetry, known as microperimetry, can be particularly useful in assessing incremental central VF and sensitivity changes [77]. Figure 3 shows an example of longitudinal changes in microperimetry in a 65-year-old CHM patient during 1 year of follow-up. At 6 and 12 months, progressive constriction of the central VF can be noted along with marked decline in retinal sensitivity. Nevertheless, VA, the most commonly used parameter to evaluate foveal function, remains unaffected (20/20).

The first gene therapy trial for CHM (ClinicalTrials.gov Identifier NCT01461213) began in 2011. The trial will assess the effects of a single subretinal injection of a recombinant adeno-associated virus (AAV) encoding the *CHM* gene. Since 2011, six patients have been injected and six more will receive the same vector, but in a 10-fold higher concentration. The aim of this study is to test the safety and tolerability of the treatment at two different doses. The secondary aim is to assess any potential functional or anatomical benefits of the treatment, comparing the treated and untreated eyes over a 24-month period. The primary outcome measure used in this study is change in VA (ETDRS [76]) at 6 months. The secondary outcome measures include changes up to 24 months in retinal sensitivity, retinal structure and area of surviving RPE as measured with microperimetry, OCT and FAF, respectively. Initial results from the first six injected patients 6 months after surgery show gains of two or more lines in patients with vision worse than 20/30 at baseline and small losses of 1 – 4 letters in patients with 20/30 or better. Retinal sensitivity has improved in all but one patient. No adverse events have been reported [78].

Additional clinical trials that will begin in 2015 (Clinical-Trials.gov identifiers NCT02077361 and NCT02341807) will test the safety and efficacy of using the same gene vector as in NCT01461213 to treat a small number of CHM male patients. Patients will receive a subretinal injection of a recombinant AAV encoding the *CHM* gene. These trials will evaluate patient safety by monitoring ocular and systemic adverse events over a 2-year period. This will be assessed by ophthalmic examinations and assays for vector dissemination and inflammation. To measure treatment efficacy, the studies will assess changes in VF and function at baseline and up to 2 years after vector delivery.

4. Therapies under investigation

4.1 Nonsense bypass therapy (Ataluren)

Approximately 30% of CHM patients carry a nonsense mutation, which results in premature termination of translation and a truncated, nonfunctional protein. One possible approach to

treat patients with nonsense mutations is via nonsense bypass therapy, a strategy that enables bypass – or read-through – of nonsense mutations during translation to allow for the translation of full-length protein. Ataluren, a drug that functions by enabling read-through of stop codons in mRNA, is currently being tested in clinical trials for cystic fibrosis (ClinicalTrials.gov identifier NCT01140451) and Duchenne muscular dystrophy (ClinicalTrials.gov identifier NCT01826487).

To date, few studies have explored nonsense bypass therapy in CHM, although there is some initial data from the CHM zebrafish model – the only CHM animal model harboring a nonsense mutation – to suggest that this therapeutic approach warrants further investigation [79].

A recent proof-of-concept report looked at the ability of Ataluren to promote translational read-through in a different retinal disease, XLRP, caused by a nonsense mutation in the *RP2* gene [80]. The research group isolated skin fibroblasts from a patient with a nonsense mutation in *RP2*, reprogrammed these cells into induced pluripotent stem cells (iPSCs), and differentiated them into RPE cells. The authors report that treating these RPE cells with Ataluren resulted in restoration of up to 20% of endogenous, full-length RP2 protein, which corresponded to reversal of known cellular phenotype defects in these cells. An earlier report found that Ataluren promoted read-through in cellular and animal models of Usher syndrome caused by a nonsense mutation [81].

4.2 AAV-mediated gene delivery

Gene transfer of *CHM* into affected CHM patients is currently being explored clinically. This approach is attractive, in part because there is a lengthy therapeutic window to preserve central vision due to the slowly progressive nature of the disease that affects rod photoreceptors first, with cone loss occurring at later stages of the disease. AAV has proven to be a valuable system for viral-based gene replacement strategies in humans, in part because AAVs cause a minimal immune response. The use of AAV-mediated gene transfer is being explored as a treatment for a number of human diseases, and results from clinical trials are showing that this therapeutic method is safe and effective in the long term – up to 3 years – in some conditions [82], including LCA [83].

Preclinical data using animal and *in vitro* models of CHM have been collected using various AAV serotypes. A 2003 study showed that REP1 expression and function could be restored in fibroblasts and lymphocytes isolated from CHM patients following AAV-mediated gene transfer *in vitro* [84]. A study published a decade later then showed that AAV serotype 2 (AAV2/2)-mediated gene transfer could lead to functional expression of REP1 in human cells *ex vivo* as well as in the retina of CHM mice [85]. In the absence of an appropriate animal model, *in vitro* human cellular models of CHM have emerged for proof-of-concept preclinical gene therapy studies. A recent study [86] that used lymphoblasts and iPSCs from CHM patients showed rescue of REP1 activity and proper protein trafficking following AAV2/2-mediated gene transfer. Disease-specific RPE cells have also been generated using the iPSC method, in an effort to avoid extrapolation from cell types not implicated in CHM pathogenesis. The AAV2/5 serotype has been shown to be superior to AAV2/2 in transducing these cells [87]. Other AAV serotypes, such as AAV2/8, have also been shown

to effectively reverse the biochemical defects in CHM both *in vitro* and in the conditional knockout CHM mouse model [88].

AAV2/2 has been extensively studied as a vector system given its tropism for several human cell types, including neurons. Indeed, a number of past, ongoing and planned clinical trials make use of AAV vector systems for gene delivery in diseases affecting the central nervous system as well as the eye [89–92]. More importantly, however, AAV2/2 has been shown to have tropism for both RPE cells and photoreceptor cells [91]; CHM is a disease in which gene replacement strategies need to target both of these cell types, making the AAV2/2 serotype particularly attractive.

AAV2/2 has been shown, in a primate model, to more effectively target rod cells than cone cells [92]. This is good news, given that rods are the primary type of photoreceptors degenerating in CHM. It is also possible, however, that AAV2/2 will transduce other ocular cell types, such as cone cells or vascular endothelial cells. Given that REP1 is ubiquitously expressed in the neuroretina, with no evidence of toxicity to the cone cells, this should not raise any concern. Taken together, the support for AAV2/2 as a vector system for gene therapy in CHM is significant. Early findings from the first gene therapy trial for CHM were recently published [78], and the data indicate general improvements in photoreceptor function following AAV2/2-mediated gene transfer. Whether or not a higher dose of the viral vector will yield better outcomes remains to be determined.

5. Expert opinion

What are the key findings and weaknesses in the research done in this field so far?

CHM is a biochemical disorder that manifests predominantly as a retinal disorder. Research to date has indicated that all components of the retina degenerate: RPE, photoreceptors and the choroidal vasculature. Key questions remain unanswered with speculation as to which cell layer is the one most affected, nominally the RPE at present. The question as to whether rods are affected more than cones remain unanswered. Does the choroidal vasculature involute as a consequence of the lack of RPE and will it respond with revascularization as a result of gene therapy directed at the photoreceptors and RPE?

What potential does this research hold? What is the ultimate goal in this field?

Research on CHM may answer some fundamental questions about the controls of vascular biology within the choroid. This research could potentially benefit the design of treatments of AMD that is affected by CNV. The ultimate goal of research on CHM is to provide a treatment to prevent vision loss.

What research or knowledge is needed to achieve this goal and what is the biggest challenge in this goal being achieved?

One of the greatest challenges of the research has been the existing animal models. The zebrafish knockout model is not viable. The knockout mouse model does not produce affected males; the carrier female is available but has limitations. Conditional knockouts in

the RPE and photoreceptors have limitations of not being a complete model. These limitations have not prevented the emergence of a gene therapy approach; however, for other experimental treatments to emerge, they will be based primarily on existing cell and animal models. The search for other models must continue if innovative approaches to treat this disorder are to be found.

Where do you see the field going in the coming years?

The following years will see expanding trials of gene replacement therapy using two similar AAV2-based REP1 vectors from NightstaRx, UK and Spark Therapeutics. These leading studies will not only benefit our understanding of the pathophysiology of CHM, but also provide evidence to support gene therapy as the standard approach to therapy for the future in patients affected by CHM. The trials will define outcome measures, other than VA, that support the selection of gene therapy as the likely most effective approach for the immediate future.

Is there any particular area of the research you are finding of interest at present?

Proof-of-concept experiments in existing cell and animal models will continue to test gene augmentation therapies in CHM. Future clinical studies will likely study the optimization of vector efficiency requiring lower doses and allowing transfection of a larger retinal area. The preclinical pipeline for other therapies such as translational bypass shows promise.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Article highlights

- Steps involved in molecular and genetic diagnosis of CHM.
- New insights into pathogenesis: Accumulation of unprenylated Rab27A in the cytosol of CHM patients results from inhibition of its prenylation by competing Rabs. No Rab-specific activity of REP1 or 2 exists.
- Recent data from animal model studies and advanced imaging in CHM patients suggest that the primary affected layer in CHM is the RPE.
- Natural history data guide patient selection and outcome measures in CHM trials. VF loss is less variable than VA as a measure to evaluate treatment outcomes.
- REP1 is ubiquitously expressed in both rods and cones in the neuroretina: potential need to transfect both photoreceptor cells in gene therapy trials.
- Overview and selection of appropriate outcome measures for clinical trials in CHM.
- Updates on AAV-mediated gene delivery: preclinical studies and gene therapy trials underway. This box summarizes key points contained in the article.

Figure 1. Fundus photographs and corresponding fundus autofluorescence (FAF) images of affected males and female carriers of choroideremia

Fundus photographs of two affected males, aged 25 (**A**) and 27 (**B**), show diffuse peripheral chorioretinal atrophy with sparing of the central macula. A resolved peripapillary CNV (white arrowhead) can be noted in (**B**). FAF imaging (**D** and **E**) demarcates the areas of remaining RPE and retina tissue in these two patients. Large choroidal vessels are visible in the absence of choriocapillaris in atrophic areas (**D**). Severe chorioretinal atrophy may unmask the underlying sclera and cause reflectance bleed-through artifact in FAF (**E**). Fundus photograph of a female carrier (47 years old) showing pigmentary mottling at the level of the RPE (**C**). FAF reveals hyper- and hypofluorescent speckles in the posterior pole (**F**).

FAF: Fundus autofluorescence.

Figure 2. Confocal immunolocalization of REP-1 in primate retina

At lower magnification (**A**), REP-1 immunolabeling (red) is visible in all layers of the neural retina. The strongest immunolabeling can be observed in the outer plexiform layer (OPL). REP-1 labeling is also prominent in the photoreceptor inner segments (IS), ganglion cell (GCL) and nerve fiber layer (NFL). Phalloidin-labeled actin staining (green) is visible in the Muller cell apical microvilli of outer limiting membrane (OLM), in OPL synapses and in retinal vessel walls. Nuclei are labeled with the DNA-binding fluorochrome DAPI. At higher magnification (**B**), the preferential labeling of cone photo-receptors is apparent. Cone pedicles (white arrows) are intensely REP-1 positive. The myoid region of the cone inner segments is more intensely labeled than the ellipsoid region of these neurons. No labeling is visible in the photoreceptor layer when anti-REP-1 antibody was omitted (**C**). GCL: Ganglion cell; IS: Inner segments; NFL: Nerve fiber layer; OLM: Outer limiting membrane; OPL: Outer plexiform layer.

Figure 3. Longitudinal microperimetry testing in choroideremia

Interpolated sensitivity color maps from the left eye of a 65-year-old CHM patient at baseline, and after 6- (B) and 12-month periods of follow-up (C). Best-corrected visual acuity was maintained at 20/20 at all visits and fixation remained foveal and stable. At baseline (A), 25 test points could be seen with stimuli dimmer than 0 dB, with a mean sensitivity of 12.88 dB. Total number of points were reduced to 22 at 6 months (B) and 13 at 12 months (C), while mean sensitivity declined to 11.60 and 7.31 dB, respectively. A color decibel scale is provided based on the manufacturer's normative data. Black dots correspond to points not seen at 0 dB.