Inherited retinal degenerations: clinical characterization on the road to therapy

Talib, M.

3.
Choroideremia

3.1

Long-term follow-up of choroideremia patients with scleral pits and tunnels as a novel observation

Sanne M. van Schuppen, MD[1*], Mays Talib, MD[2*], Arthur A. Bergen, PhD[3,4], Jacoline B. ten Brink, BAS[4], Ralph J. Florijn, PhD[4], Camiel J.F. Boon, MD, PhD[1,2], Mary J. van Schooneveld, MD, PhD[1]

Retina 2018;38(9):1713-1724

* Joint first authors.

1 Department of Ophthalmology, Academic Medical Center, Amsterdam, The Netherlands.

2 Department of Ophthalmology, Leiden University Medical Center, Leiden, The Netherlands.

3 The Netherlands Institute for Neuroscience (NIN-KNAW), Amsterdam, The Netherlands.

4 Department of Clinical Genetics, Academic Medical Center, Amsterdam, The Netherlands.

ABSTRACT

Purpose: To evaluate the long-term clinical course and visual outcome of patients with choroideremia.

Methods: Clinical examination, a social questionnaire, and medical records review of 21 patients with choroideremia from 14 families.

Results: The mean follow-up time was 25.2 years (SD: 13.3; range 2-57 years). The mean age at symptom onset was 15.1 years (SD: 10.1; range 5-40 years). Best-corrected visual acuity was stable until the age of 35 ($p = 0.96$), but declined significantly faster after the age of 35 (11%/year, $p = 0.001$), with a high variability between individual patients. The mean age at which patients discontinued working was 48.1 years (SD: 11.7, range 25-65 years). The reason for work discontinuation was vision related in 60% of cases. Most patients (70%) reported visual field constriction as the most debilitating symptom. The authors report scleral pits and tunnels as a novel finding visible on spectral domain optical coherence tomography and ophthalmoscopy.

Conclusion: Choroideremia is a severely debilitating disease showing a rapid decline of visual acuity generally after the age of 35, but a more gradual decline for other abnormalities.

INTRODUCTION

Choroideremia (CHM, OMIM 303100) is an X-linked (Xq21) progressive degeneration of the retinal pigment epithelium (RPE), outer retina, and choroid, with an estimated prevalence of 1:50.000 individuals.[1] Choroideremia is caused by loss-of-function mutations in the *CHM* gene, which encodes the Rab escort protein 1 (REP-1).[2] Affected males often start experiencing night blindness in their adolescence, a progressive visual field restriction in early adulthood, and slow or no visual acuity loss, typically until the fourth to sixth decade of life, after which visual acuity starts to decline.

It is unclear whether the disease primarily affects the choroid and RPE or the photoreceptors. Some studies in patients have suggested a primary photoreceptor disease,[3, 4] whereas other studies provide evidence that the degeneration first affects the RPE, with subsequent photoreceptor and choroidal loss.[5, 6] A recent clinical study supports this argument, showing RPE loss in early stages of disease, with subsequent loss of photoreceptor outer segments and the formation of retinal tubulations in zones of transition between intact and degenerated retina.[7] In this study, choroidal thinning occurred only after complete degeneration of the ellipsoid zone. A mouse model study has suggested that *CHM* gene mutations affect the photoreceptors and RPE independently, but because of a mutually dependant component, the degeneration occurs at an accelerated pace once both photoreceptors and RPE become progressively more dysfunctional.[8, 9]
Chorioretinal degeneration in CHM is irreversible and no treatment is available for CHM to date, but ongoing phase I/II gene therapy trials show structural and functional rescue after gene therapy.[10, 11] These results offer a promising perspective for clinical application. As gene therapy trials advance, an optimal insight into the clinical characteristics, variability, and natural disease course of CHM is needed, to define outcome measures for ongoing and future treatment trials.
Knowledge on the quality of life and social participation in affected males with CHM is currently limited, but these outcomes may become an increasingly important consideration in potential therapeutic trials. The purpose of this study was to describe the initial and longitudinal clinical characteristics of a cohort of patients with CHM, with special consideration of social participation, to gain insight into the disease's natural history.

MATERIALS AND METHODS

Study population

We ascertained 56 male probands with a clinical diagnosis of CHM from the patient database for hereditary eye diseases at the Academic Medical Center in Amsterdam (Delleman archive). Of these, 21 patients from 14 families were identified for inclusion by their present general practitioner and were included in this study. The others had moved to an unknown destination,

died, or their current general practitioner was not traceable. All 21 patients conformed to the following inclusion criteria: aged 18 years and older at the moment of clinical examination at our center, a clinical diagnosis of CHM based on a history of nyctalopia, peripheral visual field loss, and a characteristic funduscopic appearance of extensive atrophy of the RPE and choroid, made by a senior clinician with expertise in inherited retinal disease (MJS), combined with an X-linked inheritance pattern of this disease, and/or a likely disease-causing variant in the *CHM* gene. In addition, longitudinal clinical data had to be available, containing at least two measurements of best-corrected visual acuity (BCVA).

The study was approved by the Medical Ethics Committee of the Academic Medical Center in Amsterdam (the Netherlands) and adhered to the tenets of the Declaration of Helsinki. All patients provided written informed consent.

Clinical examination

Longitudinal data were obtained through a standardized review of medical records. Before clinical examination, patients were asked for age at onset of disease and at diagnosis, initial symptoms, course of complaints, family history, and overall health, through a home-sent questionnaire. Special attention was paid to socioeconomic participation in the questionnaire, enquiring on hobbies, (paid) work, driver's license, and the use of visual support devices, with a questionnaire (see Table, Supplemental Digital Content 1, which shows the topics addressed in the questionnaire and interview).

Participants were interviewed and clinically examined at the Department of Ophthalmology of the Academic Medical Center in Amsterdam, the Netherlands. Ophthalmic examinations performed included best-corrected Snellen visual acuity (BCVA) using the Early Treatment Diabetic Retinopathy Study (ETDRS) chart, central visual field tests with the Humphrey Field Analyzer (central 20° with 10-2), slit-lamp examination, ophthalmoscopy, color fundus photography, and spectral domain optical coherence tomography (SD-OCT; Topcon 3D OCT-2000, Topcon, Tokyo, Japan).

Goldmann visual field (GVF) areas of the V4e target, where available, were digitized and converted to seeing retinal areas in square millimeter using a method described by Dagnelie.[12]

Statistical analysis

Data were analyzed using SPSS version 23.0 (Version 23.0; IBM Corp, Armonk, NY). Kaplan-Meier curves for BCVA survival were used to analyze the time-to-event for the following endpoints: subnormal visual acuity in Snellen (BCVA <20/40), low vision (BCVA <20/67 and ≥20/400), and blindness (BCVA <20/400). For further analyses, BCVA was divided into the following categories based on the World Health Organization criteria: mild or no visual impairment (BCVA ≥20/67),

low vision (BCVA <20/67 and ≥20/200), and blindness (BCVA <20/400). To these categories, we added a category of "subnormal visual acuity" (BCVA ≥20/40). When visual acuity differed between two eyes, the better seeing eye was used for survival analyses.

Linear spline mixed-model analysis, used to explore rate (β) changes after a certain age, was used to evaluate the annual decline rate of BCVA, stratifying in age groups of under 35 years and ≥35 years, and converting Snellen visual acuities to logarithm of the minimum angle of resolution (logMAR), using the better seeing eye. We used the logMAR values 2.3 for counting fingers, 2.7 for hand motion, 2.8 for light perception, and 2.9 for no light perception. Simple linear regression was used to examine the association between central retinal thickness and age, after testing for linear regression assumptions.

Assessing for intra-individual symmetry in BCVA between eyes, asymmetry was defined as a difference of ≥15 ETDRS letters, which is a greater difference than the test-retest variability for visual acuity, at the last two consecutive examinations.

RESULTS

Twenty-one patients from 14 families were investigated. All patients were white males. The mean age of the participants at the time of the clinical visit was 52.9 years (SD: 15.6; range 18-73 years), and the mean age at the time of first available retrospective clinical data was 28.4 years (SD: 12.0; range 7-45 years). The mean follow-up time was 25.2 years (SD: 13.3; range 2-57 years; median 22 years), with a mean number of 5.6 visits per patient (SD: 3.3; range 2-12 visits; median 5.0). Table 1 summarizes the clinical characteristics of the patients.

Disease onset and visual acuity

The mean age at onset of the first symptom was 15.1 years (SD: 10.1; range 5-40 years), and the mean age at which an ophthalmologist was first visited was 16.5 years (SD: 9.5; range 5-39 years). In 6/21 (29%) patients, the ophthalmologist was first consulted for problems other than CHM, namely strabismus in three patients and suboptimal BCVA due to a refractive error in the three other patients. The age at disease onset was ≥10 years in 13/21 (62%) patients. Only 8/21 (38%) patients experienced their first symptom in the first decade of life. The reported first symptoms are specified in Table 2.

Although color vision difficulties were not the first reported symptoms in any of the patients, reported disturbance of color vision was present in 13/21 (62%) patients at the last visit. The mean age of patients who reported color vision complaints was 56.6 years (SD: 11.7; range 37-73 years). Patients with photoaversion (n = 18, 86%) were older (mean age 30.7, SD: 12.0, range 9-51 years)

than patients without photoaversion (mean age 19.7, SD: 17.0, range 7-39 years), although this difference was not statistically significant ($p = 0.18$, unpaired t-test).

Figure 1A shows the proportion of patients in each visual category of visual impairment against advancing age. Patients started reaching subnormal BCVA, low vision, and blindness from the fifth decade onward, with a significant trend towards worse visual categories with increasing age ($p = 0.02$, linear-by-linear association). A visual acuity of light perception was present in 6/21 (29%) patients with a mean age of 66.3 years (SD: 5.2, range 58-73 years). No patients had absence of light perception.

Kaplan Meier curves of BCVA showed a stable plateau of good vision until the fifth decade of life (Figure 1B). Mean ages for reaching subnormal vision (BCVA <20/40), low vision (BCVA <20/67 and ≥20/200), and blindness (BCVA <20/400) were 56.4 (standard error: 3.1; 95% confidence interval: 50.3-62.4), 61.3 (standard error: 2.8; 95% confidence interval: 55.9-66.8), and 65.2 (standard error: 2.7; 95% confidence interval: 59.9-70.4) years, respectively.

Figure 2A shows the BCVA at the time of examination at our clinic, showing that 7 (33%) patients between the ages of 51 and 73 were blind. The other patients, aged between 18 and 70 years were not yet blind or severely visually impaired. Figure 2B displays the course of BCVA decline with time for individual patients, generally showing a rapid BCVA decline after the age of 35 years, but with inter-individual variability, with some patients maintaining a stable BCVA well into the sixth decade of life. BCVA decline before the age of 35 was rarely seen. This age was consequently used as a spline in a linear mixed model analysis, which revealed no significant effect of age on BCVA in patients younger than 35 years ($p = 0.96$), i.e. on average a stable BCVA under the age of 35, but a significantly faster ($p = 0.001$) BCVA decline rate in patients older than 35 years of 0.045 logMAR per year ($p < 0.0001$), which translates to a yearly decline of 11% on the original Snellen scale. Although patients within the same family generally had a similar BCVA decline course, a high intrafamilial variability was observed in one family, where a patient with CHM was severely visually impaired since the age of 22 and blind since the age of 40, whereas an affected brother and cousin maintained a BCVA of 20/50 in the better seeing eye at the ages of 62 and 68, respectively.

Intra-individual asymmetry in BCVA, defined as a difference of ≥15 ETDRS letters between eyes, was found at the last two consecutive examinations in 3/21 (14%) patients. The mean BCVA was not significantly different between patients with or without an asymmetrical BCVA ($p = 0.33$, unpaired t-test), and neither was age ($p = 0.76$, unpaired t-test). In 3/7 (43%) patients, the refractive error was more myopic in the worse seeing eye. Exotropia in the worse seeing eye was noted in 3/7 (43%) patients with asymmetrical BCVA, but this was probably a consequence and not a cause of the worse BCVA, as the asymmetry in BCVA occurred at a later age in two of these patients (fifth and seventh decade of life). The other patient had a long history of asymmetry in BCVA between

eyes, and he had a large central floater in the vitreous and more foveal atrophy on SD-OCT in the worse eye.

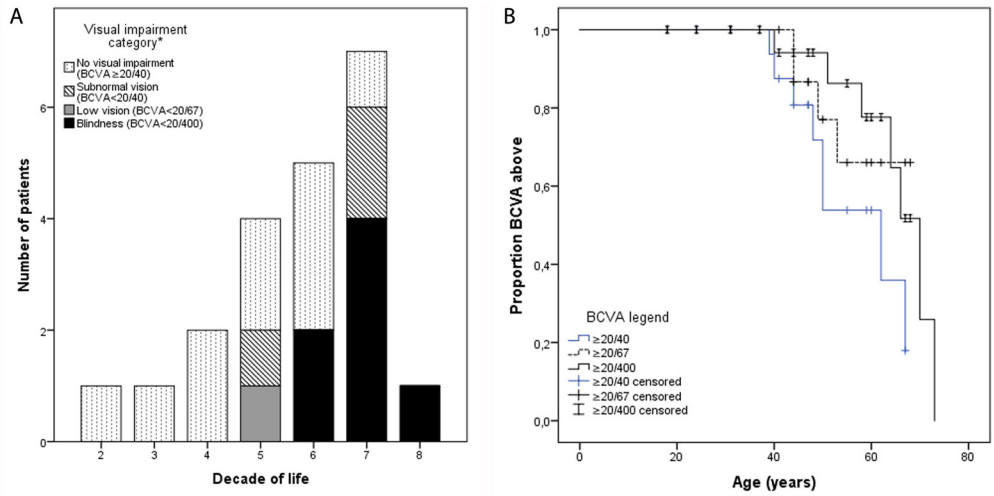

Figure 1. Age-related BCVA decline in CHM. A. Bar chart showing the number and proportion of patients in each category of visual impairment with advancing age. The BCVA and age at the last examination were used. Patients reached low vision and blindness from the fifth decade onward. **B.** Kaplan-Meier plots showing the proportion of patients with a BCVA above subnormal vision (≥20/40), above low vision (≥20/67), and above blindness (≥20/400). Censored observations, i.e. patients who had not reached the visual impairment category of interest at the last follow-up moment, are depicted as vertical bars. Median ages for reaching subnormal vision and blindness were 62.0 (standard error: 6.8; 95% confidence interval: 48.6-75.4) and 70.0 (standard error: 3.1; 95% confidence interval: 63.9-76.1) years, respectively. Although a median could not be calculated for low vision, because visual acuities rapidly declined to blindness, the curve shows that 25% of patients had low vision at the age of 50 years.

Table 1. Characteristics of individual patients with CHM at the last clinical examination

ID	Age	Eye	BCVA	Optic nerve pallor	Relative foveal sparing	Macular pigmentation	Visible vortex veins	Peripheral areas of relatively preserved retina
7	58	OD OS	LP* LP	No	Yes	Yes	Yes	Yes
9	60	OD OS	20/23 20/400	Yes	Yes	Pigment depositions along the arcades	No	No
13	24	OS OD	20/20 20/20	No	Yes	No	Yes	Yes
15	68	OS OD	20/46 20/253	Yes	Yes	No	No	Yes
18	67	OD OS	LP LP	No	No (only peripapillary remnant of retina)	No	Yes	Yes
19	62	OS OD	20/55 20/63	Yes	Yes	No	No	Yes
24	70	OD OS	LP LP	No	Yes	Yes (black granular pigment depositions)	Yes	Yes
59	51	OD OS	CF† CF	No	Yes	No	Yes	No
29	44	OD OS	20/62 20/83	No	Yes	Yes	Yes	Hyperpigmentations
30	41	OD OS	20/36 20/40	No	Yes	No	No	Yes
36	59	OD OS	20/32 20/91	Yes	Yes	No	No	Yes
38	73	OS OD	LP LP	Yes	No	No	No	Yes (only far periphery)
42	67	OS OD	20/42 20/400	No	Yes	Yes	Yes	Yes
43	47	OD OS	20/20 20/33	No	Yes	No	Yes	Yes
48	55	OD OS	20/31 20/46	No	Yes	No	Yes	Yes
51	31	OD OS	20/30 20/31	No	Yes	No	No	No
53	18	OD OS	20/20 20/400	No	Yes	No	No	Yes
54	48	OD OS	20/110 LP	No	Yes	No	Yes	Yes
57	64	OD OS	LP LP	No	Yes	No	Yes	Yes
60	37	OS OD	20/23 20/24	Yes	Yes	Yes	Yes	Yes
61	66	OS OD	LP LP	No	Yes	No	Yes	Yes

CF, counting fingers; LP, light perception vision.

Ophthalmic and funduscopic findings

A myopic refractive error was found in 20/21 (95%) patients (Table 2). Cataract was reported in 18/20 (90%) patients (mean age 54.2 years, SD: 14.1, range 18-70 years). These patients were significantly older than the two patients who had a clear lens in both eyes at the ages of 24 and 37 years, respectively (p = 0.034, unpaired t-test). Of the patients with cataract, six had undergone uncomplicated cataract surgery (mean age 57.8 years, SD: 8.1, range 44-68 years). In the remaining 12 patients with cataract, the predominant cataract type was posterior subcapsular in four (33%) patients, nuclear in one (8%) patient, both posterior subcapsular and nuclear in five (42%) patients, anterior subcapsular in one (8%) patient, and star shaped in one (8%) patient.

All patients showed the characteristic appearance of a whitish fundus with large areas of profound atrophy of the choroid and retina (Figure 3). The optic disc appearance was normal in 16/21 (76%) patients. Scattered coarse hyperpigmentations in the atrophic fundus were seen in 19/21 (90%) and not mentioned or documented in 2/21 (10%) patients. In 15/21 (71%) patients, a small island of RPE and retina (relative foveal sparing) persisted in the fovea and/or perifoveal area. In 4/21 (19%) patients, small islands of relatively preserved RPE persisted outside the vascular arcade. In 13/21 patients (62%), large choroidal vessels or vortex veins in the periphery were visible on fundoscopy. In 7/21 patients (33%), small excavations or "pits" in the sclera were visible on fundoscopy, either in the macula (2/21, 10%) or at the border of the posterior pole and in the midperiphery (5/21, 24%) (Figure 4). From some pits, a vessel emerged (Figure 4). Peripapillary atrophy was found in one (5%) patient.

Table 2. Cohort characteristics of patients with CHM

Characteristics	
Nystagmoid eye movements, n (%)	6/19 (32)
Photophobia, n (%)	18 (86)
Reported first symptom, n	
Nyctalopia, n (%)	17 (81)
Subjective visual acuity loss, n (%)	3 (14)
Photophobia, n (%)	1 (5)
Subjective visual field loss, n (%)	-
Subjective color vision loss, n (%)	-
Mean refractive error (SER) ±SD, D (range)	-3.17±2.6 (-9.63 – 0.5)
SER ≤-6D, n (%)	5 (24)
-2D ≥ SER > -6D, n (%)	8 (38)
0D ≥ SER > -2D, n (%)	7 (33)
2D ≥ SER > 0D, n (%)	1 (5)
Shallow anterior chamber, n (%)	9 (43)
Fundoscopic examination	
Optic disc pallor, n (%)	6 (29)
Vascular attenuation, n (%)	13 (62)

SER, spherical equivalent of the refractive error.

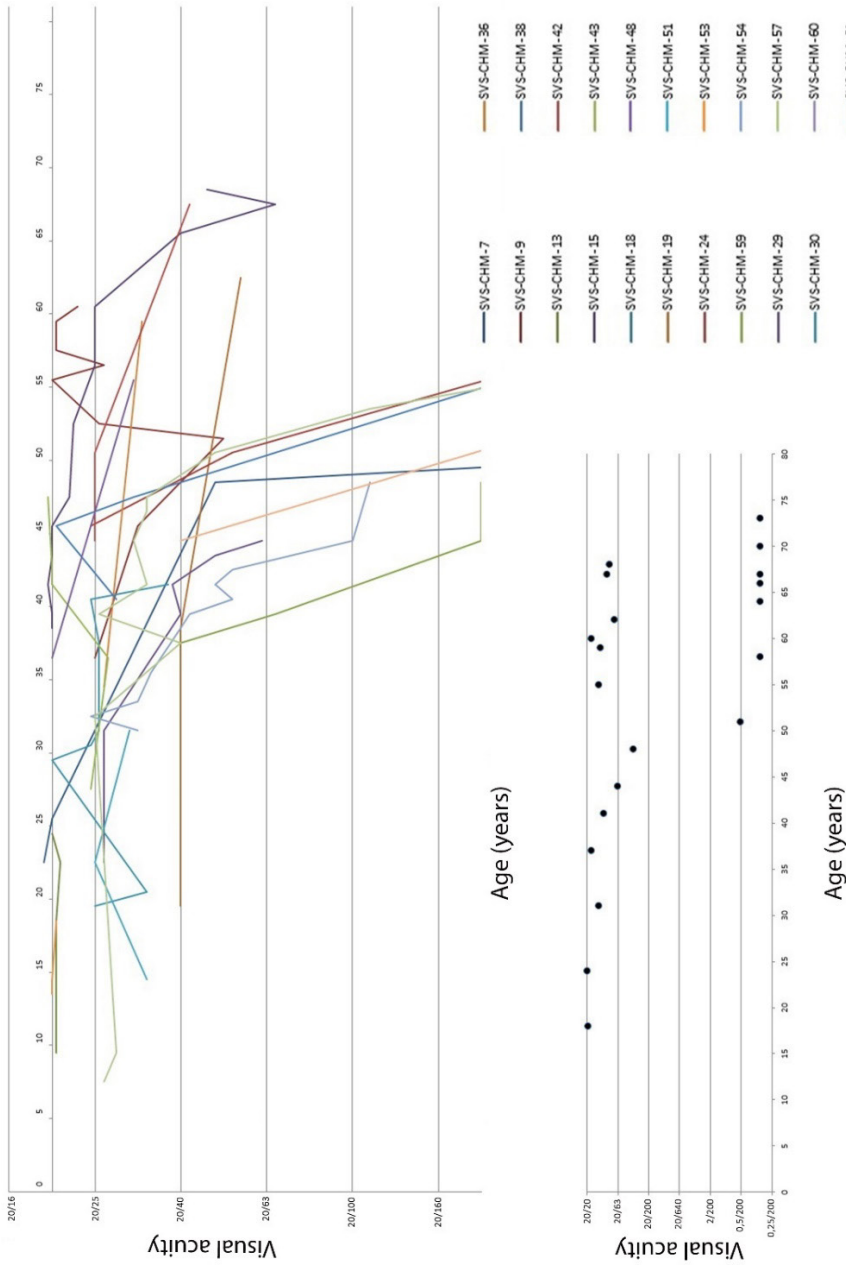

Figure 2. Visual acuities of individual participants. A. BCVA of individuals at the final clinical examination. **B.** The course of BCVA decline in individual participants. Although some individuals (n = 8) showed a rapid decline in BCVA within the age range of 35-50 years, others showed a slower decline in BCVA from this age onward. Some patients showed a (temporary) increase in BCVA after cataract surgery or a certain degree of BCVA measurement variability.

Findings on retinal imaging

The SD-OCT images were available for all patients, but because of eccentric or insufficient fixation and decentered images, measurements of the central retinal thickness could be performed in 17/21 (81%) cases (n of eyes = 29). OCT images of 13/21 (62%) patients showed retinal thinning of all layers with foveal atrophy. Four patients (19%) aged 18 to 37 years had a fully intact foveal ellipsoid zone (Figure 4), whereas the other patients, aged 41 to 70, had a fragmented or absent ellipsoid zone. The mean central retinal thickness was 140 μm (SD: 67, range 54-273 μm). Simple linear regression revealed a significant correlation between central retinal thickness and age at examination (p = 0.007), with 35% of the variance in central retinal thickness explained by age (adjusted R^2 = 0.35). When assessing the fundus and SD-OCT images of individual eyes (total n = 42), we found that all eyes with low vision (BCVA <20/67) or blindness (BCVA <20/400) based on the BCVA at the last examination (n = 22) showed atrophy of all retinal layers in the macula on SD-OCT and/or profound macular chorioretinal atrophy on fundus photography. In eyes with mild BCVA impairment (BCVA <20/40) at the last examination (n = 6), at least a small area of central relative preservation of the RPE and retinal layers was visible on SD-OCT and/or fundus photography. In patients who had no visual acuity impairment (BCVA ≥20/40) at the last examination (n = 14), preservation of the RPE and retinal layers in the macula was visible on SD-OCT and fundus photography in 13/14 (93%) patients, and in one patient, no SD-OCT or fundus photography of the better seeing eye was present.

Scleral tunnels were visible on OCT in 13/21 (62%) patients. On fundoscopy, these features seemed a pit, but OCT showed in nine patients (43%) an interruption of the sclera, RPE, and the remaining choroid at the location of the pit (Figure 4), whereas a scleral tunnel under an uninterrupted RPE was visible in eight patients (38%), with some patients having both scleral pits and tunnels. In one patient, a scleral tunnel emerged into an interruption of the choroid and RPE. Multiple outer retinal tubulations were found in the outer nuclear layer in 16/21 (76%) patients, aged between 18 and 70 years (Figure 4), who had visual acuities ranging between 20/20 and light perception (n = 4). In 10/21 (48%) patients, aged between 18 and 67 years, intraretinal cystoid fluid collections were present in the outer (10/10, 100%) and inner retina (3/10, 30%) (Figure 4). An epiretinal membrane was found in 13/21 (62%) patients. In two patients (10%) with an epiretinal membrane and cystoid fluid collections, aged 44 and 51 years, a full-thickness macular hole was seen on OCT (Figure 4). One other 41-year old patient with an epiretinal membrane had a lamellar macular hole.

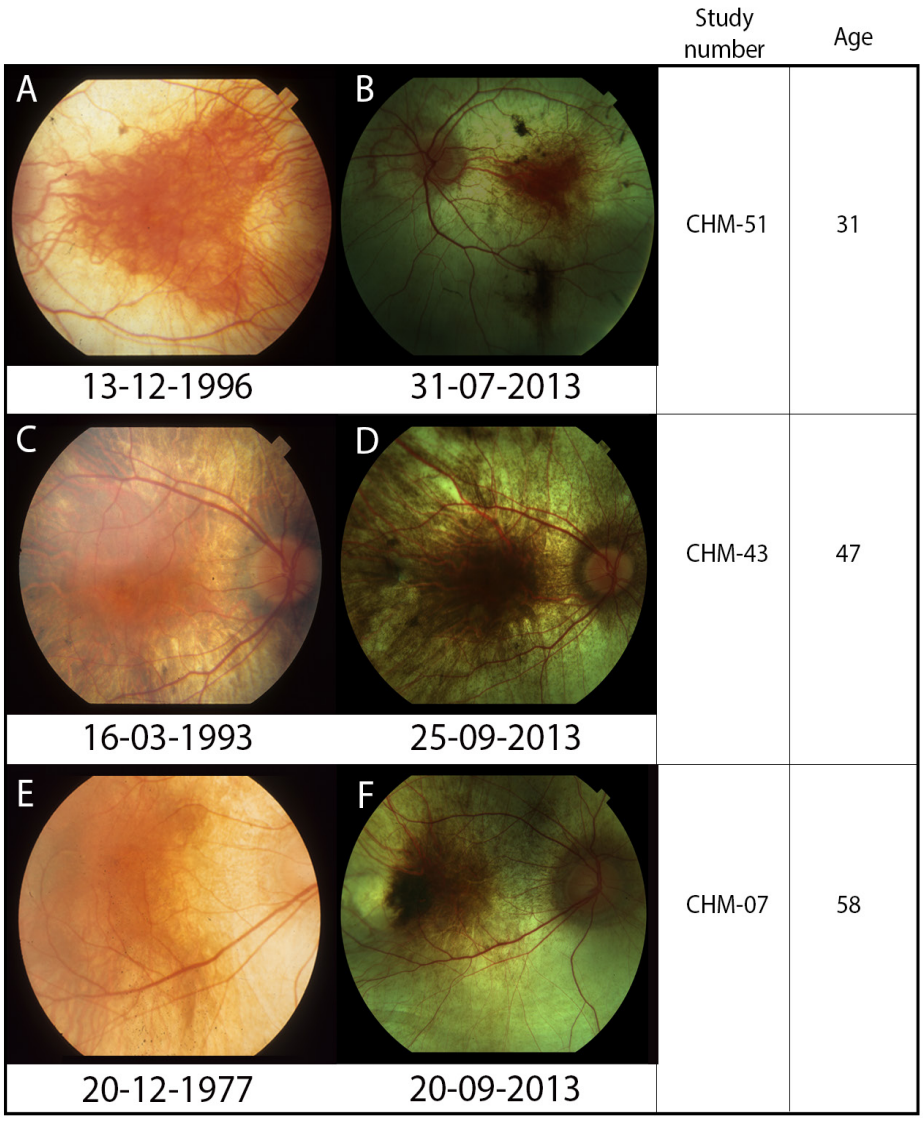

Figure 3. Fundoscopic images of patients with CHM. The first column (**A, C,** and **E**) shows fundus photographs of patients in the past and the date on which the image was taken. The second column (**C, D,** and **F**) shows a fundus photograph taken during the study examination and the date of this visit. The third and fourth columns display the study number and age at the last examination, respectively. Patients show mild vascular attenuation, progression of chorioretinal atrophy, and scattered coarse hyperpigmentations in the posterior pole. The optic disc maintains a relatively normal color and aspect.

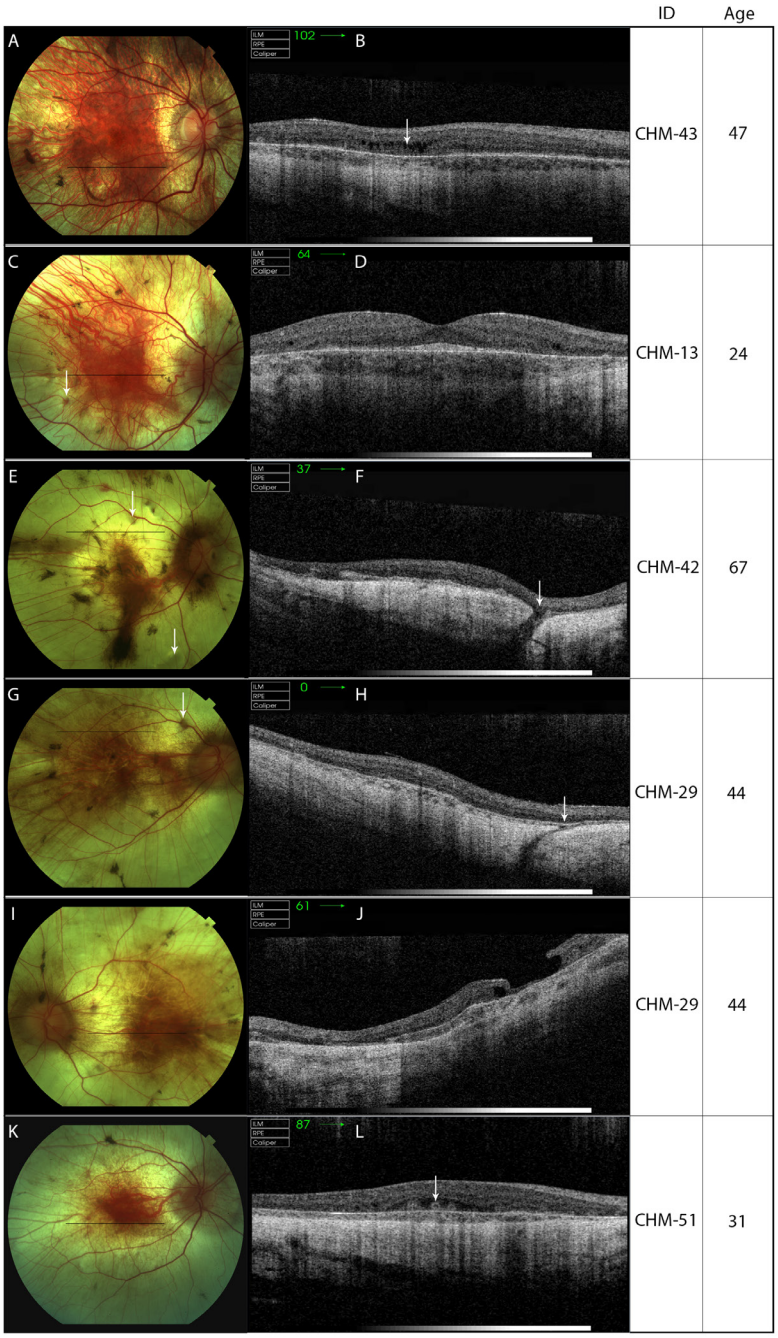

Figure 4. SD-OCT images of patients with choroideremia. The first column shows fundus photographs with typical findings in choroideremia. The black lines indicate the location of the corresponding SD-OCT image. The second column shows the corresponding SD-OCT scans, and the third and fourth column show the patient study number and age at examination, respectively. **A** and **B.** Fundus photograph and SD-OCT image of a patient with macular

cystoid fluid collections (arrow) in the outer retinal layers. **C** and **D.** Imaging of a patient with a scleral pit (**C**, white arrow) and relative foveal sparing on fundus photography, the latter corresponding with preservation of the ellipsoid zone, external limiting membrane and outer nuclear layer on SD-OCT. The peripheral macula showed attenuation of these layers, and cystoid fluid collections. **E** and **F.** Imaging of a patient with scleral pits on fundus photography (**E**, white arrows) and a scleral tunnel (**F**, white arrow) with interruption of the sclera, remaining choroid, and retinal pigment epithelium (RPE) on SD-OCT. **G** and **H.** A patient with a scleral pit on fundus photography (**G**, white arrow) and a scleral tunnel under an uninterrupted RPE layer on SD-OCT (**H**, white arrow). **I** and **J.** A patient with a full-thickness macular hole, visible on SD-OCT. **K** and **L.** Imaging of a patient with outer retinal tubulations in the outer nuclear layer on SD-OCT (**L**, white arrow).

Visual field findings

GVFs (n = 28) were available for seven patients. The median age at the first available GVF was 32.0 years (range 14.0-62.0 years). Within individuals with follow-up GVFs (n = 5; median follow-up time 7.0 years, range 2.0-17.0 years), seeing retinal areas, averaged between eyes, declined in size with a mean slope of decline of 22.0 mm^2/year, corresponding with approximately 20° visual field diameter per year (SD: 10.4 mm^2/year, range 4.8-30.3 mm^2/year, corresponding with 9°-23°/year). There were various patterns of remaining visual field: 4/7 patients, aged 36 to 62 years, had a central island with one or multiple mainly temporal remnants of variable sizes; one patient, aged 30, had a midperipheral scotoma; one patient, aged 47 only had a central island; and the youngest patient, aged 14, had a fully intact GVF for the V4e isopter. Reliable visual field testing of the central 20° (10-2) was only possible in five patients (24%; n of eyes = 9), because central BCVA and fixation were too impaired to allow for reliable central visual field testing in the other patients. Patients in whom reliable central visual field testing could be performed had relative foveal sparing and were younger (mean 44.2 years, SD: 16.3, range 24-60 years) than patients in whom it could not be reliably performed (mean age 55.7, SD: 14.8, range 18-73 years), although this age difference was not statistically significant ($p = 0.15$, unpaired *t*-test). With the exception of one 24-year-old patient with a virtually normal central visual field, static perimetry revealed sharply delineated absolute parafoveal scotomas with low foveal sensitivity (mean 26.6 dB, SD: 10.8, range 8.0-35.5 dB). No correlation between age and foveal sensitivity could be established in this cohort. The mean deviation was -26.9 dB (SD: 7.3, range -33.2 to -14.92 dB, $p < 1\%$ in all tested eyes). The mean pattern standard deviation was 8.3 dB (SD: 3.4, range 3.7-12.2, $p < 1\%$ in all tested eyes). The mean central visual field size in horizontal diameter was 8.7° (SD: 3.8, range 4.5°-14°).

Social participation

All patients completed a questionnaire which contained a section dedicated to social participation and were interviewed on this topic. Table 3 displays these questions and the patients' responses. The mean age at which patients discontinued working, was 48.1 years (SD: 11.7, range 25-65 years). Of the patients who had obtained a driver's license in the past, only one, aged 39, was still able to drive. Patients who used visual aid devices (17/21, 71%) were significantly older (mean 58.2 years, SD: 11.1, range 31-73 years) than patients who did not need visual aid devices (mean 30.5 years,

Table 3. Social topics addressed in the questionnaire

Topic adressed				
1.	Still working	6/21 (29%) yes	15/21 (71%) no	
2.	Reason to stop working	9/15 (60%) vision-related	6/15 (40%) other (reorganization, retirement)	Not applicable in 6/21 (29%), still working
3.	Reason not to work full-time	6/21 (29%) vision-related	15/21 (71%) work(-ed) fulltime	
4.	Most debilitating symptom in daily life	14/20 (70%) visual field constriction	3/20 (15%) blindness	2/20 (10%) visual acuity or 1/10 (5%) other symptom
5.	Reading ability	7/21 (33.3%) without visual aid	7/21 (33%) with visual aids	7/21 (33%) not able to read anymore
6.	Driver's license in the past	10/21 (47.6%) yes	11/21 (52.4%) never	
7.	Use of visual aid devices	17/21 (71%) yes	4/21 (19%) no	
	a. Speech output system for computer	8/21 (38%) yes	12/21 (57%) no	1/21 (5%) unknown
	b. Cane	9/21 (43%) yes	11/21 (53%) no	1/21 (5%) unknown
	c. Braille	4/21 (19%) yes	16/21 (76%) no	1/21 (5%) unknown
	d. Guide dog	2/21 (10%) yes	18/21 (86%) no	1/21 (5%) unknown
8.	Smoking	7/20 (35%) yes	2/20 (10%) smoked in past	11/20 (55%) never
9.	Alcohol consumption	18/20 (90%) yes	2/20 (10%) no	
10.	Other ophthalmologic diagnoses	7/21 (33%) yes (6/7 cataract, 1/7 Charles-Bonnet syndrome)	14/21 (67%) no	
11.	Chronic systemic diseases	6/20 (30%) yes	14/20 (70%) no	

SD: 11.3, range 18-41 years) ($p < 0.001$, unpaired *t*-test). Of the patients who reported subjective peripheral visual field loss as the most debilitating symptom (14/20, 70%), 11 /14 (79%) had normal or mildly subnormal BCVA, 1/14 (7%) had low vision, and 2/14 (14%) were legally blind. Of the eight patients with low vision or blindness, 3/8 (38%) found subjective peripheral visual field loss the most debilitating symptom despite the central visual impairment. The other patients with visual impairment reported visual acuity complaints or blindness as the most debilitating symptom. Interviews with patients generally revealed increased dependence on their spouse or partner due to their CHM-related debilitations, often leading to tension in the relationship due to increased responsibilities of the patient's partner. Four patients (19%) were currently living alone and had no children. All patients were still able to commute independently, using aid devices such as a cane.

DISCUSSION

This work provides a cross-sectional and retrospective analysis of the age-related BCVA changes and clinical characteristics in CHM. These results extend the previous observations about the natural disease course in patients with CHM, in the wake of gene therapeutic trials showing promising results and the surging demand for more careful phenotyping and longitudinal follow-up of patients with CHM.[10]

The long mean follow-up of 25.2 years and the standardized clinical work-up in this study allowed for comprehensive analysis of the natural course of the BCVA in this patient group. To the best of our knowledge, this is the longest reported follow-up of a CHM cohort. The rate of disease progression remains a key question in the context of therapeutic outcome measures and prognostic counselling. In this study a rapid loss of BCVA rarely occurred before the age of 35 years, which is consistent with previous findings of a correlation of BCVA decline with age only after 30 and 40 years of age.[13, 14] Our observed annual BCVA decline of 11% (0.045 logMAR units per year) was similar to a recent finding by Freund et al,[14] but faster than the previously documented 0.007 to 0.0206 logMAR units.[15, 16] The high degree of intra-individual symmetry in BCVA supports the use of the contralateral eye as a nontreated control in ongoing and future gene therapy trials.

The visual field loss was severe in this cohort, with a mean seeing retinal area decline rate of 22.0 mm^2/year, or 20° visual field diameter per year, and a mean remaining horizontal central visual field size of 8.7° at the mean age of 44 years, although this was measured in a small group of patients (n = 5) with ages ranging between 14 and 62 years. Previously, Freund et al found a biphasic model of visual field loss, with a critical age at 20 years, and high interindividual variability below that age. As ongoing gene therapy trials for retinal dystrophies have mainly focused on the central retina, static threshold perimetry and microperimetry results have been important in evaluating the therapeutic

outcome in gene therapy for *RPE65*-related retinal dystrophies.[10, 17] The small sample size and the relatively old age of our cohort were limitations to establishing an accurate relationship between age and visual field size, and a preferably prospective longitudinal analysis of visual field size in a larger group of patients is necessary, particularly as results of recent studies suggest that visual field may be at least as important as BCVA as a tool for evaluating treatment efficacy in trials.[14] Of note, current gene therapeutic strategies use subretinal injection in the macula of an adenoviral vector carrying the normal *CHM* gene,[10] and a gene therapy effect in this case could be only expected on parameters reflecting macular function such as BCVA and central retinal sensitivity.

Although we did not perform standardized color vision testing during the clinical work-up, subjective reports of color vision complaints in 62% of this cohort support the earlier suggestions that color vision should be part of the clinical workup of patients with CHM.[13, 18] However, as we did not perform color vision testing, we cannot conclude whether color vision defects are an early symptom in CHM and could be of prognostic value.

Scleral features in the form of interruptions or tunnels were visible in a large proportion (13/21; 62%) in our study. To our knowledge, such areas have not been previously described in patients with CHM, and we coin the term "scleral pits" when there was a clear interruption of the sclera, choroid and RPE, and used the term "scleral tunnel" in the case of an uninterrupted RPE. The significance of this finding is unclear, because in individuals without choroidal atrophy these scleral tunnels may also be present, but masked by the intact choroid and/or RPE. Possibly, these scleral areas may correspond to zones of sclera-perforating blood vessels and scleral emissaries in which these blood vessels have become progressively atrophic, leaving the scleral pits and tunnels that harbored this vasculature. The presence of outer retinal tubulations, previously described as round or ovoid hyporeflective spaces with hyperreflective margins and usually located in the outer nuclear layer on SD-OCT,[19, 20] in this cohort is an interesting finding, because previous studies using SD-OCT have reported the presence of outer retinal tubulations in CHM patients in varying numbers, ranging from no mention to 91% of eyes.[7, 13, 21] These structures are typically found in advanced age-related macular degeneration and other degenerative retinal conditions, and although the underlying pathogenesis remains unknown, they are postulated to result from a tubular rearrangement of degenerating photoreceptors.[19] Previous studies showed no relation of the presence of these structures with age,[13, 19] or with findings on fundus photography.[22] However, outer retinal tubulations have been found to be present around areas of surviving retina, and could therefore be clues of areas retaining some visual function.[7] In some patients in our cohort, these tubulations were found near areas of more intact photoreceptor layers on SD-OCT, although this finding was not consistently present and four patients had only light perception vision in both eyes, suggesting that in our study there is no clear relation between visual acuity and the presence of outer retinal tubulations. We also identified epiretinal membrane in the macula in 11/20 (55%) patients with CHM who underwent OCT imaging. Also, patients with CHM may

be at an increased risk of developing a macular hole. If clinically significant, macular hole and/or epiretinal membrane can be treated with vitrectomy, inner limiting membrane peeling, and gas tamponade.[23] Areas of outer retinal tubulations, macular holes, epiretinal membrane, and retinal thinning in or near the fovea may increase the risk of complications during and after subretinal gene therapy administration, and may influence the functional outcome of gene therapy. Even after anatomically and/or functionally successful surgical treatment of macular hole and/or epiretinal membrane, the macula may still be more vulnerably during gene therapy surgery, for instance in terms of an increased risk of re-opening a macular hole during submacular gene therapy vector delivery. These findings, including the finding of scleral pits and tunnels, warrant further investigation, using longitudinal SD-OCT images in larger patient populations.

A unique feature of our study was the focus on the disease influence on social participation in these patients. Patients generally remained professionally active well into the fifth decade of life and nearly half (48%) of patients obtained a driver's license, although only one patient still retained a driver's license at the time of the last examination. Current gene therapeutic trials focus on parameters reflecting central retinal function, but 70% of patients in our study indicated that they experienced peripheral visual field restriction as the most debilitating factor in their daily life. In patients with visual impairment, the proportion experiencing peripheral visual field restriction as the most debilitating symptom was 38%. Future therapeutic strategies for CHM may therefore be aimed at a timely intervention targeting not only the central retina, but also the peripheral retina in an attempt to prevent or stop a decline in peripheral visual function. As ongoing gene therapy trials in patients with CHM have shown clinically promising results, the effect on social participation may become an increasingly important outcome measure to evaluate.

The limitations of the current study include the relatively small sample size and the lack of peripheral visual field data. We did not find genotype-phenotype correlations in this CHM cohort, in part because subgroup analysis is impeded by the inadequate sample size, and as previous studies have shown no genotype-phenotype correlations, probably due the virtually complete lack of REP-1 protein expression in CHM, independent of the causative mutation.[14, 24, 25] Future studies investigating the social participation in patients with an inherited retinal dystrophy could use a comprehensive, standardized and validated questionnaire, adapted for a visually impaired population.[26, 27]

In conclusion, patients with CHM in this cohort had a sudden and rapid BCVA decline that first started between the ages of 35 and 60 years, with scleral pits or tunnels as a novel common finding. Longitudinal follow-up of other outcome measures, such as visual field decline, central sensitivity, and structural retinal changes, is necessary to evaluate efficacy of therapeutic options.

REFERENCES

1. MacDonald IM, Sereda C, McTaggart K, Mah D. Choroideremia gene testing. Expert Rev Mol Diagn 2004;4:478-484.
2. van den Hurk JA, Schwartz M, van Bokhoven H, et al. Molecular basis of choroideremia (CHM): mutations involving the Rab escort protein-1 (REP-1) gene. Hum Mutat 1997;9:110-117.
3. Jacobson SG, Cideciyan AV, Sumaroka A, et al. Remodeling of the human retina in choroideremia: rab escort protein 1 (REP-1) mutations. Invest Ophthalmol Vis Sci 2006;47:4113-4120.
4. Syed N, Smith JE, John SK, et al. Evaluation of retinal photoreceptors and pigment epithelium in a female carrier of choroideremia. Ophthalmology 2001;108:711-720.
5. Mura M, Sereda C, Jablonski MM, et al. Clinical and functional findings in choroideremia due to complete deletion of the CHM gene. Arch Ophthalmol 2007;125:1107-1113.
6. Flannery JG, Bird AC, Farber DB, et al. A histopathologic study of a choroideremia carrier. Invest Ophthalmol Vis Sci 1990;31:229-236.
7. Xue K, Oldani M, Jolly JK, et al. Correlation of Optical Coherence Tomography and Autofluorescence in the Outer Retina and Choroid of Patients With Choroideremia. Invest Ophthalmol Vis Sci 2016;57:3674-3684.
8. Tolmachova T, Anders R, Abrink M, et al. Independent degeneration of photoreceptors and retinal pigment epithelium in conditional knockout mouse models of choroideremia. J Clin Invest 2006;116:386-394.
9. Tolmachova T, Wavre-Shapton ST, Barnard AR, et al. Retinal pigment epithelium defects accelerate photoreceptor degeneration in cell type-specific knockout mouse models of choroideremia. Invest Ophthalmol Vis Sci 2010;51:4913-4920.
10. MacLaren RE, Groppe M, Barnard AR, et al. Retinal gene therapy in patients with choroideremia: initial findings from a phase 1/2 clinical trial. Lancet 2014;383:1129-1137.
11. Edwards TL, Jolly JK, Groppe M, et al. Visual Acuity after Retinal Gene Therapy for Choroideremia. N Engl J Med 2016;374:1996-1998.
12. Dagnelie G. Conversion of planimetric visual field data into solid angles and retinal areas. Clin Vis Sci 1990;5:95-100.
13. Heon E, Alabduljalil T, Iii DB, et al. Visual Function and Central Retinal Structure in Choroideremia. Invest Ophthalmol Vis Sci 2016;57:377-387.
14. Freund PR, Sergeev YV, MacDonald IM. Analysis of a large choroideremia dataset does not suggest a preference for inclusion of certain genotypes in future trials of gene therapy. Mol Genet Genomic Med 2016;4:344-358.
15. Roberts MF, Fishman GA, Roberts DK, et al. Retrospective, longitudinal, and cross sectional study of visual acuity impairment in choroideraemia. Br J Ophthalmol 2002;86:658-662.
16. Coussa RG, Kim J, Traboulsi EI. Choroideremia: effect of age on visual acuity in patients and female carriers. Ophthalmic Genet 2012;33:66-73.
17. Bainbridge JW, Mehat MS, Sundaram V, et al. Long-term effect of gene therapy on Leber's congenital amaurosis. N Engl J Med 2015;372:1887-1897.
18. Jolly JK, Groppe M, Birks J, et al. Functional Defects in Color Vision in Patients With Choroideremia. Am J of Ophthalmol 2015;160:822-831.e3.

19. Zweifel SA, Engelbert M, Laud K, et al. Outer retinal tubulation: a novel optical coherence tomography finding. Arch Ophthalmol 2009;127:1596-1602.
20. Goldberg NR, Greenberg JP, Laud K, et al. Outer retinal tubulation in degenerative retinal disorders. Retina 2013;33:1871-1876.
21. Khan KN, Islam F, Moore AT, Michaelides M. Clinical and Genetic Features of Choroideremia in Childhood. Ophthalmology 2016;123:2158-2165.
22. Iriyama A, Aihara Y, Yanagi Y. Outer retinal tubulation in inherited retinal degenerative disease. Retina 2013;33:1462-1465.
23. Zinkernagel MS, Groppe M, MacLaren RE. Macular hole surgery in patients with end-stage choroideremia. Ophthalmology 2013;120:1592-1596.
24. Esposito G, De Falco F, Tinto N, et al. Comprehensive mutation analysis (20 families) of the choroideremia gene reveals a missense variant that prevents the binding of REP1 with Rab geranylgeranyl transferase. Hum Mutat 2011;32:1460-1469.
25. Simunovic MP, Jolly JK, Xue K, et al. The Spectrum of CHM Gene Mutations in Choroideremia and Their Relationship to Clinical Phenotype. Invest Ophthalmol Vis Sci 2016;57:6033-6039.
26. Massof RW, Ahmadian L, Grover LL, et al. The Activity Inventory: an adaptive visual function questionnaire. Optom Vis Sci 2007;84:763-774.
27. Bruijning JE, van Rens G, Fick M, et al. Longitudinal observation, evaluation and interpretation of coping with mental (emotional) health in low vision rehabilitation using the Dutch ICF Activity Inventory. Health Qual Life Outcomes 2014;12:182.

SUPPLEMENTAL MATERIAL

Supplemental Digital Content 1. Topics addressed in the questionnaire and the interview

Topics addressed in questionnaire and interview
Ophthalmic history; other ophthalmological diagnoses
Age at onset symptoms
Subjective symptoms and debilitating effect on daily life; nyctalopia, complaints of visual field, visual acuity, color vision, or other complaints
Ethnicity
Reading abilities
Professional life
Educational levels obtained
Employment, reasons for unemployment if applicable
Driver's license
Use of visual aid devices
General health
Chronic/systemic diseases, allergies
Surgery ocular/non-ocular
Smoking/alcohol consumption/drug use
Medication history
Family history of choroideremia
Family situation
Civil status
Children
Effect of choroideremia on relationships; dependency on partner