Achromatopsia (ACHM) is an autosomal recessive cone dysfunction disorder affecting approximately one in 30,000 live births worldwide and is characterized by lack of function of all three classes of cone photoreceptor. Six causative genes are considered responsible for over 90% of cases: CNGA3, CNGB3, GNAT2, PDE6H, PDE6C, and ATF6. Affected individuals display marked photophobia and pendular nystagmus from birth or early infancy, associated with reduced visual acuity, deficient or absent color vision, and central nystagmus from birth or early infancy, associated with reduced visual acuity, deficient or absent color vision, and central nystagmus. At present, there are no curative treatments available, with current management aiming to minimize the effects of symptoms and optimize residual visual function. However, promising results from studies investigating gene-replacement therapy to restore cone function in animal models with ACHM has led to the recent establishment of a number of human clinical trials, evaluating the safety and efficacy of gene supplementation in ACHM. Assessment of retinal structure and knowledge of the natural history of ACHM is key to identifying subjects potentially suitable for this intervention and also for subsequently monitoring the effects of treatment. ACHM has classically been described as a stationary condition. Sundaram et al. evaluated 40 individuals with ACHM aged between 6 and 52 years, and found no significant correlation of deterioration in visual acuity, contrast sensitivity, or reading acuity with advancing age. Optical coherence tomography (OCT) has demonstrated variable outer retinal structure at the central macula, including at the level of the photoreceptor inner segment ellipsoid layer (ISe) and retinal pigment epithelium (RPE). In their study, Sundaram et al. found that the variable spectral-domain OCT (SD-OCT) appearances did not correlate with age or genotype and that total foveal retinal thickness (FTRT), foveal outer nuclear layer (ONL) thickness, and ISe intensity were not age-dependent. Given these results supporting a stationary natural history, the authors purported that there may potentially be a wider window for therapeutic intervention. In contrast, other work has alluded to progressive cone photoreceptor loss in ACHM. However, these studies have often been limited by their cross-sectional nature and small sample size. To address this, a larger prospective structural and functional ACHM study (n = 38) has been conducted by Aboshiha et al. with the aim of performing statistical analysis on the data acquired to draw more definitive conclusions about the frequency and rate of any disease progression. The authors reported essentially stable SD-OCT findings over time. There is a relative paucity of studies investigating fundus autofluorescence (FAF) in ACHM and, in
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particular, over time. Therefore, Aboshiha et al. additionally investigated this parameter. The authors concluded that the retinal parameters they assessed either remained stable or changed minimally in a small proportion of patients. However, it is not inconceivable that monitoring patients over a longer period may identify a greater proportion of individuals who develop progressive changes. Since an understanding of the natural history of ACHM is key to the planning and selection of appropriate candidates for such therapeutic interventions as gene therapy, we have performed serial assessment of SD-OCT and FAF in a longitudinal study of subjects with molecularly proven ACHM, with a larger cohort size and longer follow-up duration than previously conducted. Our aim was to further examine structural progression in ACHM, thereby making inferences about the optimal timing for treatment.

METHODS

Subjects

Fifty subjects with ACHM, characterized phenotypically and genotypically, were prospectively seen between 32 and 86 months after baseline assessment. All subjects were participants of the ACHM natural history study conducted at Moorfields Eye Hospital. The study protocol adhered to the tenets of the Declaration of Helsinki and was approved by the Moorfields Eye Hospital Ethics Committee. Informed consent was obtained from all subjects prior to their entry to the study.

Clinical Assessments

Best-corrected visual acuity (BCVA) and contrast sensitivity were measured at baseline and follow-up assessments using an Early Treatment Diabetic Retinopathy Study chart and Pelli-Robson chart at 1 meter, respectively.

Spectral-Domain OCT (SD-OCT)

SD-OCT imaging was performed on both eyes following pupillary dilation with tropicamide 1% and phenylephrine 2.5% eye drops. Line and volume scans were acquired with the Spectralis device (Heidelberg Engineering, Inc., Heidelberg, Germany) using the same protocol as that employed by Aboshiha et al. At follow-up visits, the device was switched to follow-up mode so the same scanning location was used both at baseline and follow-up. This enabled comparable measurements to be made between the two visits for a given subject but may not have been entirely possible in individuals with significant nystagmus. The transverse scale of each image was calculated using the axial length of the corresponding eye. This was measured using the Zeiss IOL Master (Carl Zeiss Meditec, Inc., Jena, Germany) using the same protocol as that employed by Sundaram et al. Using the AF mode on the Spectralis device (Heidelberg Engineering) following pupillary dilation as previously described and after SD-OCT image acquisition. In subjects displaying a well-demarcated region of reduced autofluorescence at both visits, the area was measured by tracing around the circumference of the hypoautofluorescent region with a mouse-driven cursor and recording the area within, as calculated by the in-built image analysis software (Heidelberg Eye Explorer, Heidelberg Engineering). All FAF measurements were made by the same examiner (NH). For each FAF image, the mean of three area measurements was calculated and used for further analysis.

Data Analysis Methods

The D’Agostino-Pearson Omnibus Test was used to verify the normality of data before using any parametric tests. The distribution of FTRT, ONL thickness, and HRZ diameter values did not demonstrate normality; therefore, nonparametric tests were used throughout for statistical analysis. Statistical tests were performed using GraphPad Prism, version 5 (GraphPad Software, Inc., La Jolla, CA, USA). Qualitative changes in foveal structure on SD-OCT were evaluated in both eyes for each subject. Sundaram et al. found no significant differences in measured parameters between the two eyes of subjects in their cross-sectional study of ACHM, and therefore selected the left eye for further quantitative analysis; this was replicated by Aboshiha et al. in their subsequent longitudinal study. In keeping with these previous studies, we also utilized the left eye for more detailed analysis of quantitative parameters.

RESULTS

Fifty patients with greater than 24 months follow-up who had interpretable transfoveal SD-OCT scans at baseline and follow-up were included in the study. Twenty-six patients were male (52%) and 24 were female (48%). The mean age of the cohort at baseline was 23.9 years (range, 7–52 years). Forty-five subjects (90%) had baseline and follow-up FAF. Of these 50 subjects, 32 had previously been described by Sundaram et al. Table 1 summarizes the age at baseline, follow-up interval, and structural parameters in our cohort over time. Supplementary Table S1 cross-references our subjects to those included in the study by Sundaram et al. and demonstrates the detailed genotype of each patient. Supplementary Table S2 shows the absolute FTRT and ONL measurements for the left eye of each subject, at baseline and follow-up.

SD-OCT

Comparable foveal SD-OCT scans were obtained at baseline and follow-up in all 50 subjects. Six of these subjects (12%) showed qualitative progression bilaterally on SD-OCT between baseline and follow-up. Patients 2, 24, 37, and 38 progressed from category 1 (continuous IS layer) at baseline to category 2...
Thirty-seven subjects (74%) showed foveal hypoplasia bilaterally at baseline, and no change in the presence or absence of this feature in either eye was found between baseline and follow-up in any patient.
FTRT, ONL Thickness and HRZ Diameter

FTRT was measured in all 50 subjects at baseline and follow-up. Mean change in absolute FTRT (i.e., FTRT at follow-up minus FTRT at baseline) was 0.94 μm (range, −10 to +9 μm). The change in absolute FTRT between visits in the cohort was not statistically significant (Wilcoxon signed-rank test; \( P = 0.2459 \)). Mean percentage change in FTRT between baseline and follow-up was −0.98% (range, −8.55% to +6.12%). The percentage change in FTRT within the cohort was also not statistically significant (Wilcoxon signed-rank test; \( P = 0.0732 \)). There was no significant correlation between age and change in FTRT (Spearman’s correlation coefficient = −0.17; \( P = 0.2336 \)). There was, however, a positive correlation between starting FTRT and change in FTRT, where subjects with a lower FTRT at baseline showed greater reduction over time (Spearman’s correlation coefficient = 0.41; \( P = 0.0029 \)). This suggests that the less the initial FTRT, the greater the degree of central retinal thinning over time.

Three (8%) of the 37 subjects with foveal hypoplasia (patients 10, 28, and 46) did not have a structurally distinct ONL that could be accurately measured and were excluded from ONL thickness analysis. The mean change in absolute ONL thickness in the remaining 47 subjects (i.e., ONL thickness at follow-up minus ONL thickness at baseline) was +1.98 μm (range, −10 to +8 μm). This change was statistically significant between assessments (Wilcoxon signed-rank test; \( P = 0.0084 \)). Mean percentage change in ONL thickness between baseline and follow-up was +2.19% (range, −14.29% to +8.08%). The percentage change in ONL thickness within the cohort was also statistically significant (Wilcoxon signed-rank test; \( P = 0.0031 \)).

Five of the 12 patients who had an HRZ at both baseline and follow-up had a starting absolute FTRT greater than the average of the entire cohort. There was no statistically significant change in the diameter of the HRZ over a mean follow-up interval of 67 months among these 12 patients (Wilcoxon signed-rank test; \( P = 0.3737 \)). Mean change in absolute FTRT
within this smaller cohort was $-0.50 \mu m$ (range, $-7$ to $+5 \mu m$), that is, smaller than the mean reduction in absolute FTRT noted in the entire cohort over time.

Figure 3 demonstrates the range of changes in structural parameters, observed over time within our cohort.

**FAF**

Forty-five subjects (90%) had baseline and follow-up FAF (Table 1). As in the study by Aboshiha et al., three distinct FAF patterns were observed in our subjects at baseline: (1) a normal FAF pattern, seen in 14 patients (31%); (2) an abnormal
central increase in FAF, seen in 13 patients (29%); and (3) a well-demarcated central reduction in FAF, seen in 18 patients (40%). No subject demonstrated a change in FAF pattern over time. The area of reduced FAF was measured at baseline and follow-up in those individuals demonstrating this phenotype, since this feature was amenable to quantification. Ten of these subjects had a starting absolute FTRT smaller than the average of the entire cohort. Mean increase in the area of reduced FAF was 0.055 mm², over a mean follow-up interval of 43 months. The change in FAF between baseline and follow-up was statistically significant (Wilcoxon signed-rank test; \( P = 0.0011 \)).

Mean change in absolute FTRT within the 18 subjects demonstrating reduced FAF was \(-0.78 \mu m\) (range, \(-9\) to \(+9 \mu m\)), smaller than the mean reduction in absolute FTRT noted in the entire cohort over time.

**Genotype-Phenotype Correlation**

\( CNGA3 \) and \( CNGB3 \) subgroups were analyzed separately, and no statistically significant difference was noted between the two cohorts regarding change in FTRT (Mann-Whitney \( U \) test; \( P = 0.5187 \)) or change in ONL thickness (Mann-Whitney \( U \) test; \( P = 0.5986 \)). Comparisons could not be made with \( GNAT2 \), \( PDE6C \), and \( ATF6 \) subgroups, given the small numbers of individuals within these groups. Similarly, statistical comparisons could not be made between \( CNGA3 \) and \( CNGB3 \)
subgroups regarding change in HRZ diameter and area of reduced FAF, again due to small sample sizes.

**BCVA and Contrast Sensitivity**

Table 2 displays the BCVA and contrast sensitivities of the subjects at baseline and follow-up. For consistency, only data from the left eye were analyzed. The mean BCVA at baseline was 0.91 logMAR (range, 0.68–1.32 logMAR), and this was significantly different from the mean BCVA at follow-up, which measured 0.87 logMAR (range, 0.60–1.20 logMAR; paired t-test; \( P = 0.014 \)). However, the mean of the logarithm of contrast sensitivity (logCS) at baseline of 1.16 logCS (range, 0.5–1.55 logCS) was not significantly different from that measured at follow-up of 1.15 logCS (range, 0.25–1.65 logCS; paired t-test; \( P = 0.681 \)), where higher logCS values indicate better contrast sensitivity.

**DISCUSSION**

The natural history of ACHM has been the center of some debate, with several studies suggesting it is a progressive...
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condition. Thiadens et al. investigated 40 subjects with achromatopsia cross-sectionally with SD-OCT and proposed that cone cell decay began in early childhood, with retinal thinning correlating with age. Thomas et al. found in their study of 15 individuals with ACHM that the presence of a HRZ and a fovea were also age dependent. However, this study was limited by its cross-sectional nature. To identify cone loss over time, there is a need for longitudinal assessment.

Thomas et al. followed 8 subjects with ACHM over a mean period of 16 months. They found that the 5 younger subjects (>10 years) showed progressive morphological change in the 30 subjects for whom this parameter was assessed. In those subjects with a reduced FAF, Aboshiha and colleagues did not observe a statistically significant change in HRZ diameter in those subjects where this feature was present at baseline and follow-up visits. With regard to FAF, Aboshiha et al. sought to address this with their longitudinal investigation of 38 subjects with ACHM. They reported retinal structure to be largely stable over time regarding SD-OCT. Only 2 subjects showed qualitative evidence of progression, as evidenced by advancing OCT grade bilaterally. There was no statistically significant change in the quantitative SD-OCT parameters assessed, for instance, FTRT and ONL thickness. Furthermore, there was no statistically significant change in HRZ diameter in the 9 subjects with an HRZ at baseline and follow-up visits. With regard to FAF, Aboshiha and colleagues did not observe a change in FAF pattern between baseline and follow-up visits, or between eyes, in the 30 subjects for whom this parameter could be assessed. In those subjects with a reduced FAF pattern at baseline and follow-up (n = 15), a small but statistically significant reduction in the median area of reduced FAF was noted between visits. However, the mean follow-up period of their cohort was 19.5 months (range, 13–24 months), and it is possible that retinal change may occur over longer periods.

More recently, and consistent with our data, Triantafylla et al. reported no changes in FTRT and only minor changes in ONL thickness in their study of 10 subjects with ACHM, with a mean follow-up period of 65.9 months (Triantafylla et al., IOVS 2018;59:ARVO E-Abstract 5205). However, they reported a 2.5-fold increase in HRZ width, which we did not observe in our data. Interestingly, 6 of their 10 patients were children under the age of 10, while 7 of our 50 subjects were 10 years old or younger. Thus, we cannot rule out the idea that there is some progression very early in the disease that stabilizes later in the first decade. Consistent with this, the 2 subjects in our study with the greatest ONL thickness change were 10 and 11 years of age. This highlights the need to begin natural history studies in even younger populations as well as the availability of handheld OCT as useful in these efforts.

To our knowledge, our work represents the largest longitudinal study of retinal structure in ACHM. The large patient sample size allows for statistical analysis of the significance of any change over time. Our cohort showed no statistically significant change in FTRT over time nor in HRZ diameter in those subjects where this feature was present at the baseline assessment. This is consistent with the results of the longitudinal study undertaken by Aboshiha et al. and supports the notion that ACHM is a predominantly stable condition with regard to retinal structure. The increase in ONL thickness over time was statistically significant. However, this change was small (less than 2 μm on average). Thus, while the change in this parameter may be statistically significant, its clinical relevance is questionable. Indeed, retinal thickness has been shown to change with age. Nieves-Moreno et al. reported a reduction in retinal thickness for each year of increased age in healthy subjects, concluding that the thickness of most retinal layers varies with age. Our measurements of change in retinal thickness are within those expected for normal aging, suggesting that they do not represent true disease progression. Nevertheless, the change was also age dependent. However, the sample size of our study was limited and the number of those with a decrease in thickness was small, and statistical significance of any change over time. Our cohort showed no statistically significant change in FTRT over time nor in HRZ diameter in those subjects where this feature was present at baseline and follow-up visits. With regard to FAF, we identified three distinct patterns in our cohort, as per previous work. The Spectralis SD-OCT device (Heidelberg Engineering) sets the baseline scan as the reference scan, and the in-built software attempts to direct the SD-OCT fixation target to a position that corresponds to the same retinal location during subsequent image acquisition. However, this is not always possible in ACHM patients with severe nystagmus and poor fixation. A follow-up scan may therefore be centered at a slightly different position, and this may contribute subtle variations in the quantitative and qualitative results recorded compared to baseline. This suggests that there may be a role for collecting multiple representative scans at baseline.

In showing qualitative change, we reported a higher proportion of subjects (12%) than did the work of Aboshiha et al., who described this in 5% of their cohort. However, their study had a mean follow-up period of only 19.5 months and fewer subjects (38 compared to our 50). By contrast, the subjects in our study showing qualitative change all had follow-up periods of greater than 40 months. Therefore, it may be that foveal appearances do gradually deteriorate in a small proportion of patients, given sufficient time. Further work assessing retinal function in patients who have been followed-up over extended periods may shed light on any potential functional consequences of such changes. Aboshiha et al. found that patients demonstrating qualitative change did not show significant associated changes in functional parameters. SD-OCT changes, however, may predate change in visual function, and extended follow-up could allow these delayed functional changes to manifest.

With regard to FAF, we identified three distinct patterns in our cohort, as per previous work. The three patterns were present in relatively equal proportions among our cohort and did not change in any subject between baseline and follow-up. In those patients with reduced FAF at baseline, there was a statistically significant increase in the area of reduced signal over time; however, this rate of increase was very small (0.015 mm² per year). This is very similar to the rate of 0.02 mm² per year identified by Aboshiha et al. A range of retinal mechanisms can result in variations in the observed FAF...
pattern. Of note, our results show no particular relationship between the observed pattern of FAF and qualitative SD-OCT appearances for subjects with SD-OCT categories 1 through 4. Thus, all three FAF patterns were observed across these categories. However, all 3 subjects with SD-OCT category 5 scan at baseline and follow-up demonstrated a pattern of reduced FAF. This is because of the atrophic retinal changes seen in this SD-OCT phenotype, which in turn are associated with a reduced density of the fluorophore lipofuscin in the affected regions. Finer quantitative methods of FAF analysis are available,12,14 and it may be that further work using these techniques may identify more subtle changes in FAF patterns. However, the clinical significance of FAF in ACHM remains poorly established, and we would predict that any progression in this parameter is likely to be slow and small in magnitude. Interestingly, recent work by Matet et al.15 has advocated the role of near-infrared autofluorescence (NIR-AF) in the investigation of ACHM. While the Spectralis device performs short-wavelength autofluorescence (SW-AF) imaging, NIR-AF is a less frequently used imaging modality. In subjects with ACHM, the authors correlated NIR-AF with SW-AF features and structural alterations on SD-OCT. Patients with a central hypo-autofluorescence on NIR-AF were older and displayed more advanced structural abnormalities at the fovea than did those with normal NIR-AF, while SW-AF was not correlated to other multimodal findings. This suggests a potential role for NIR-AF as an imaging biomarker in ACHM.15

In summary, our findings from a larger cohort and longer follow-up of individuals with ACHM provide further support to the presiding notion that this is essentially a stable condition regarding retinal structure and that any change over time is likely to be small, slow, and occur in only a limited proportion of patients. Of note, Zein et al.16 recently studied the effect of ciliary neurotrophic factor (CNTF) in 5 subjects with CNGB3-ACHM, all of whom had an HRZ on SD-OCT at baseline. CNTF is thought to have a neurotrophic effect on human cone photoreceptors. The group demonstrated a sustained filling or collapse of the HRZ in all treated eyes. They proposed that CNTF was responsible for the changes in retinal foveal morphology since they were not seen in untreated eyes. Our findings lend support to the idea that marked changes in retinal morphology observed following the therapeutic interventions tested to date can be attributed to the treatment rather than to disease progression since we have demonstrated that structural changes in the natural history are negligible and very slow.

Further work using more detailed imaging techniques, such as adaptive optics scanning light ophthalmoscopy (AOSLO), may better demonstrate the earliest indicators of any structural progression in ACHM.17 AOSLO allows visualization of individual rod and cone cells in vivo18,19 and has confirmed the presence of cone photoreceptors in all patients with ACHM, albeit reduced in number compared to the unaffected retina, with a wide range of cone densities, and often a disconnect with SD-OCT appearance.20 With regard to assessing progression of ACHM with AOSLO, Langlo et al.21 demonstrated that foveal cone structure showed little or no change in their cohort of patients with CNGB3-ACHM over a follow-up period of 6 to 26 months. However, extending follow-up (as in our study of SD-OCT and FAF imaging) may provide new insights into very gradual retinal changes occurring at the microscopic level.

Interestingly, while Aboshiha et al.9 demonstrated stability of functional parameters in conjunction with structural stability, the results from our cohort show a small but clinically significant improvement in mean BCVA over time, though no significant change in contrast sensitivity. The change in BCVA may partially be explained by the fact that 7 subjects within our cohort were age 10 years or younger at presentation. In these individuals, the transition from childhood toward adulthood over prolonged follow-up may have enabled more accurate assessment of visual acuity at follow-up, thus leading to an apparent improvement in this parameter. Furthermore, nystagmus in children with ACHM may lessen with age.22 The improved fixation associated with this could potentially lead to a mild improvement in visual acuity. Nevertheless, while the increase in BCVA within our cohort over time was statistically significant, it was small in magnitude and thus likely to be of minimal clinical significance.

For now, our work suggests that disease progression for the majority of our cohort is negligible. Thus, there may potentially be a wide window for therapeutic intervention, and gene replacement therapy may be a viable option for affected adults as well as children. However, the functional benefit derived by older individuals may be limited by abnormal organization of the visual cortex. Baseler et al.23 described reorganization of the visual cortex from birth onward in ACHM in response to abnormal visual experience. The authors attributed this to visual system plasticity, postulating that this may result from a range of mechanisms, including reorganization of neural connectivity and changes in the efficacy of established connections. Plasticity of the brain is maximal in the first few years of life, reducing with advancing years.24 Thus, while it is conceivable that older subjects may show some improvement following treatment, it is likely that earlier intervention in children, when anomalous visual processing pathways are less developed, may yield optimal results. Our work supporting the largely nonprogressive nature of ACHM is also valuable when evaluating the effects of potential interventions in a clinical trial setting. Knowledge of the condition’s relative stability enables structural and functional changes occurring post-treatment to be attributed to the intervention rather than to the natural disease process, allowing for better recognition of the specific effects of the treatment itself. Indeed, trial regulatory bodies will often seek evidence confirming the disease’s stationary nature prior to approving a study, and our results may aid in the establishment of future interventional studies for ACHM.

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