



# Report



## Retinal Degeneration in Choroideremia follows an Exponential Decay Function

Fundus autofluorescence (AF) arises from lipofuscin, which is derived from retinoid byproducts of the visual cycle and accumulates within retinal pigment epithelial cells. Alterations in AF pattern are seen in a wide range of retinal degenerations. Choroideremia is an X-linked retinal dystrophy caused by loss-of-function mutations within the *CHM* gene, encoding Rab escort protein-1.<sup>1</sup> It is uniquely characterized by a central “island” of residual AF that undergoes gradual shrinkage with disease progression. The decrease in AF area is correlated precisely with loss of overlying photoreceptors, leading to progressive visual field restriction and blindness around the fifth decade. Retinal gene replacement therapy using an adeno-associated viral vector could potentially slow down or stop disease progression in choroideremia.<sup>2,3</sup> Although visual acuity may be improved by gene therapy, it is affected relatively late in the disease; therefore, the area of residual AF may provide an alternative anatomic biomarker for monitoring progression at earlier stages. A previous cross-sectional study suggested an exponential decrease in AF area with age in choroideremia.<sup>4</sup> However, longitudinal data on natural disease progression is lacking. For instance, it is uncertain whether individuals progressed at different rates depending on genetic, epigenetic, or environmental factors, and whether the rate of progression varied between early and late stages of the disease.

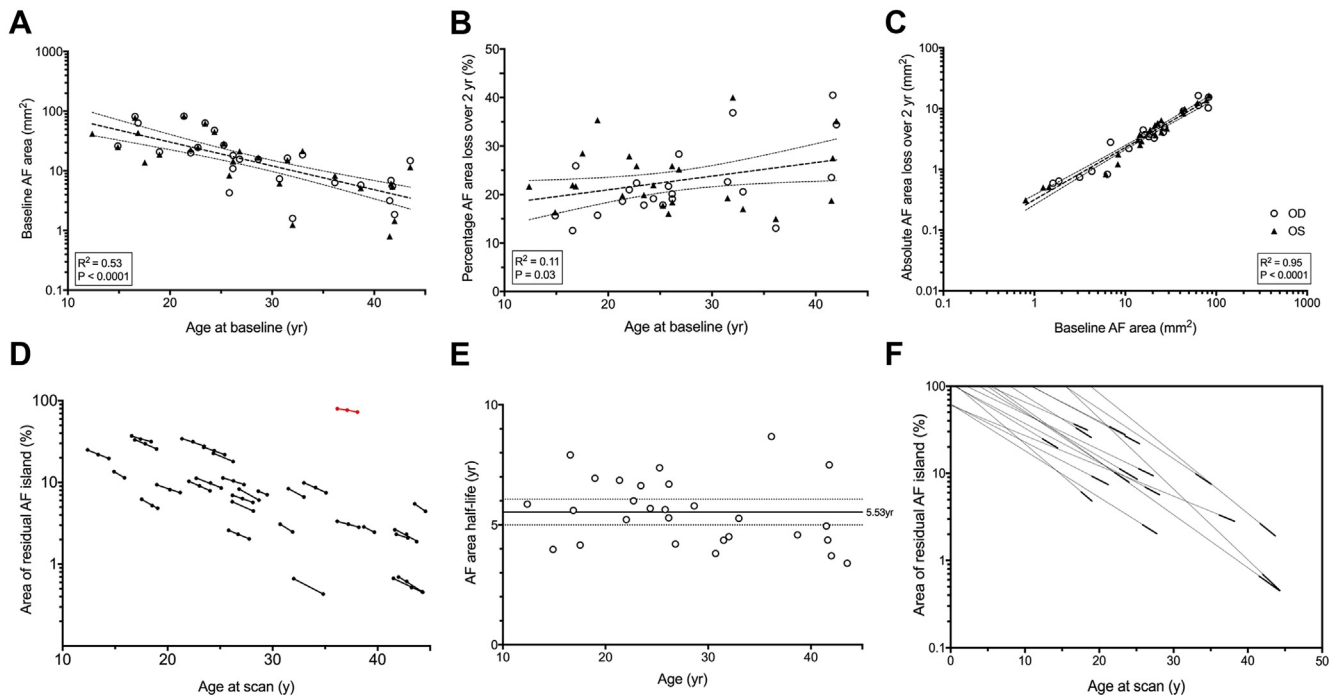
We performed a retrospective, longitudinal analysis of AF images from 62 eyes of the youngest 31 confirmed patients with choroideremia (mean age, 28 years; range, 12–43 years), who have relatively large and therefore measurable AF areas, and were seen as part of the screening process for gene therapy trials approved by the UK Research Ethics Committee (ref: GTAC171 & 15/LO/1379; Table S1, available at [www.aaojournal.org](http://www.aaojournal.org)). BluePeak 55° AF images were captured on the Spectralis confocal scanning laser ophthalmoscope (Heidelberg Engineering, Heidelberg, Germany) at baseline, and 1 and 2 years. The central contiguous AF island was outlined manually using the area tool within Heidelberg Eye Explorer (HEYEX) by 2 independent graders (J.W.A. and J.C.W.) to provide a mean. Area outlines from follow-up visits were transferred onto the baseline image using the internal image registration function of HEYEX, thereby overcoming discrepancies arising from small changes in focus, orientation, and edge distortion.

Correlation between baseline AF area and age suggested an exponential decline of AF area over time ( $R^2 = 0.5$ ;  $P < 0.0001$ ;  $n = 56$  eyes), consistent with a previous report (Fig 1A).<sup>4</sup> The percentage of AF area loss over 2 years in each eye showed a weak correlation with age (slope, 0.28;  $R^2 = 0.11$ ;  $P = 0.03$ ;  $n = 44$  eyes; Fig 1B). The absolute AF area loss over 2

years was, however, strongly correlated with AF area at baseline ( $R^2 = 0.97$ ;  $P < 0.0001$ ;  $n = 44$  eyes; Fig 1C). Because age and baseline AF area are not independent variables in a progressive retinal degeneration, multiple regression analysis was performed. This analysis showed a strong overall correlation between AF area loss over 2 years and baseline AF area and age ( $R^2 = 0.94$ ;  $P < 0.0001$ ;  $n = 44$  eyes). However, age alone was not a significant correlate ( $R = -0.005$ ; 95% confidence interval,  $-0.01$  to  $0.00$ ;  $P = 0.06$ ), whereas baseline AF area was a highly significant positive correlate ( $R = 0.81$ ; 95% confidence interval,  $0.72$ – $0.89$ ;  $P < 0.0001$ ). Altogether, the rate of AF area reduction seemed to depend on the baseline area, with larger areas progressing more quickly, in keeping with exponential decay. Hence, the slowing of degeneration in later years is due to the small baseline area remaining rather than any protective effect of ageing.

If AF area undergoes steady exponential decay, the rate of degeneration in the first year should predict the rate of degeneration in the second, which seemed indeed to be the case (Fig 1D). Because a good degree of intereye symmetry was observed (paired  $t$ -test:  $P = 0.53$ ;  $t = 0.64$ ; degrees of freedom [df] = 20;  $n = 21$  patients), the AF data from both eyes were combined to calculate the AF half-life in each individual (Fig 1E). The mean AF area half-life for the cohort was 5.53 years (95% confidence interval, 5.0–6.1). Therefore, the exponential decay constant ( $\lambda$ ) was 0.125, using the equation  $N(t) = N(0)e^{-\lambda t}$ . No correlation was found between AF half-life and age ( $P = 0.2$ ;  $n = 28$ ), further confirming that the rate of disease progression is independent of age. Our estimate of AF half-life is slightly shorter than that predicted by the cross-sectional study (8.7 years).<sup>4</sup> A possible explanation is that the latter extrapolated half-life from a regression line fitted to the whole cohort and constrained to the maximum measurable area of the 55° field at age zero. However, it can take several variable years before the AF defects progress to within the central 55° field. We propose an alternative model, whereby the degeneration of AF follows a “one-hit” model such that the probability of retinal pigment epithelial/photoreceptor cell death remains constant over time. The implication is that the time of onset of detectable AF loss may be the critical factor that determines eventual disease severity, because once the degeneration starts, it progresses at a similar exponential rate. By extrapolating individual half-lives, the range of age of onset of AF degeneration involving the 55° posterior pole were predicted to be 0 to 20 years (Fig 1F). Our human data are consistent with the kinetics of photoreceptor degeneration in mouse models of retinitis pigmentosa, which followed exponential decreases with similar  $\lambda$  constants.<sup>5</sup>

The data suggest that the majority of patients with choroideremia undergo retinal degeneration with a similar rate of exponential decay. Possible modifiers that may account for some of the



**Figure 1.** The residual area of autofluorescence (AF) in choroideremia undergoes exponential decline. Sixty-two eyes of 31 patients with choroideremia were included. The AF images were either of poor clarity ( $n = 5$ ) or unavailable at one of the timepoints ( $n = 6$ ), in which case only the 2 remaining timepoints were analyzed. The right eyes of 2 patients (7 and 24) were excluded owing to poor quality images. In addition, patients 2 and 17 were included in the analysis of baseline AF area, but excluded from progression analysis because parts of their preserved AF islands extended beyond the edge of the  $55^\circ$  field, thus preventing accurate measurement of progression. **A**, The correlation between residual AF area ( $\text{mm}^2$ , shown on a log scale) and age in a cohort of 56 eyes (28 patients). Similar patterns were seen for the right (hollow circles) and left (solid triangles) eyes. **B**, A weak correlation was seen between percentage AF area loss over 2 years and age at baseline ( $R^2 = 0.11$ ;  $P = 0.03$ ;  $n = 44$  eyes). **C**, A strong linear correlation was seen between absolute AF area loss over 2 years and AF area at baseline (both on a log scale;  $R^2 = 0.97$ ;  $P < 0.0001$ ;  $n = 44$  eyes). In **A–C**, the dashed lines are the regression lines and the dotted lines represent 95% confidence intervals of the regressions. **D**, The mean AF area of both eyes in each patient (percent of the total  $55^\circ$  field;  $n = 29$  patients) was plotted against age (in years to 2 decimal places) with connecting lines between dots indicating area changes over 1 to 2 years. P2 (the late starter) is superimposed (in red) for comparison but excluded from the progression calculation as mentioned. **E**, In total, 23 patients had useable images over a 2-year period and 5 had useable images for  $< 2$  years. The AF area half-life of these patients did not show correlation with age ( $R^2 = 0.06$ ;  $P = 0.2$ ;  $n = 28$  patients). The solid line represents the mean half-life for the cohort (5.53 years) and the dotted lines represent the 95% confidence interval of the mean (4.99–6.07 years). **F**, Predicting the age of disease onset based on the rate of AF progression. The mean AF area of both eyes of each individual (shown as percentage of the total  $55^\circ$  field on a log scale) was plotted against age (in years, calculated to 2 decimal places). The solid lines represent linear regressions of AF area trends in each individual seen in **D**. Backward extrapolation of the gradients predicts the age at which the degeneration may have started to involve the  $55^\circ$  posterior pole, assuming a constant exponential decay (dotted lines). Only patients with AF area measurements at 3 time-points were included ( $n = 18$ ) because a half-life calculation based on only 2 time-points would be more liable to error. In 3 individuals, the predicted age of onset was negative. This finding likely reflects the variability in the accuracy of half-life estimates.

variation in AF half-life are area measurement errors, and variability in developmental anatomy of the retina/choroid and AF island shapes. Bland-Altman analysis of test–retest variability in AF area measurements between our 2 graders showed a 95% limits of agreement of  $-0.80$  to  $0.68 \text{ mm}^2$  ( $n = 22$ ) and bias of  $-0.06$  (standard deviation, 0.38). Comparisons of the shapes of the AF islands between fellow eyes revealed a remarkable degree of mirror symmetry and islands with more “craggy” outlines seemed to shrink at a slightly higher rate (Fig S1, available online at [www.aaojournal.org](http://www.aaojournal.org)). Overall, the results support the use of fellow eyes as controls in clinical trials, particularly when baseline AF areas are symmetrical. Alternatively, individuals can be observed for a period of time to determine their baseline AF half-life before a therapeutic intervention is given to 1 eye.

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## Report

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