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ORIGINAL ARTICLE

# Investigating the impact of asymmetric macular sensitivity on visual acuity chart reading in choroideremia

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## Abstract

**Introduction:** Degeneration in choroideremia, unlike typical centripetal photoreceptor degenerations, is centred temporal to the fovea. Once the fovea is affected, the nasal visual field (temporal retina) is relatively spared, and the preferred retinal locus shifts temporally. Therefore, when reading left to right, only the right eye reads into a scotoma. We investigate how this unique property affects the ability to read an eye chart.

**Methods:** Standard- and low-luminance visual acuity (VA) for right and left eyes were measured with the Early Treatment of Diabetic Retinopathy Study (ETDRS) chart. Letters in each line were labelled by column position. The numbers of letter errors for each position across the whole chart were summed to produce total column error scores for each participant. Macular sensitivity was assessed using microperimetry. Central sensitivity asymmetry was determined by the temporal-versus-nasal central macular difference and subsequently correlated to a weighted ETDRS column error score. Healthy volunteers and participants with X-linked retinitis pigmentosa GTPase regulator associated *retinitis pigmentosa* (*RPGR*-RP) were used as controls.

**Results:** Thirty-nine choroideremia participants (median age 44.9 years [IQR 35.7–53.5]), 23 *RPGR*-RP participants (median age 30.8 years [IQR 26.5–40.5]) and 35 healthy controls (median age 23.8 years [IQR 20.3–29.0]) were examined. In choroideremia, standard VA in the right eye showed significantly greater ETDRS column errors on the temporal side compared with the nasal side ($p = 0.002$). This significantly correlated with greater asymmetry in temporal-versus-nasal central macular sensitivity ($p = 0.04$). No significant patterns in ETDRS column errors or central macular sensitivity were seen in the choroideremia left eyes, nor in *RPGR*-RP and control eyes.

**Conclusion:** Difficulty in tracking across lines during ETDRS VA testing may cause excess errors independent of true VA. VA assessment with single-letter optotype systems may be more suitable, particularly for patients with choroideremia, and potentially other retinal diseases with asymmetric central macular sensitivity or large central scotomas including geographic atrophy.

**KEYWORDS**
ETDRS chart, inherited retinal disease, macular sensitivity, visual acuity

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## INTRODUCTION

The Early Treatment of Diabetic Retinopathy Study (ETDRS) visual acuity (VA) chart was designed in 1982, incorporating the principal design features of the Bailey–Lovie Log MAR chart, and has since led to the standardisation of VA measurement in clinical trials.[1,2] Visual acuity testing with the ETDRS chart is quick and easy to implement as well as being relatively low-cost. The logarithmic progression of letter sizes, with an equal number of letters per line and equal spacing, improves the consistency of VA measurements, while the cumulative counting scoring system for ETDRS VA (i.e., the number of letters read) is more amenable to statistical analyses compared to both Snellen scoring or Log MAR units.[3,4] Furthermore, the ETDRS chart provides a greater range of larger letters enabling more reliable VA assessment in individuals with low vision compared to the Snellen chart in both normal and low luminance. For these reasons, VA assessment using the ETDRS chart and letter scoring has become a leading outcome measure in many ophthalmic clinical trials.[5]

Although the superior accuracy and reproducibility of the ETDRS chart, compared to other charts, are widely documented,[6] VA measurement with the ETDRS chart is not without its limitations. First, VA only reflects the visual function of the central 0.5° of the macula.[7] Second, ETDRS testing lacks a forced choice paradigm, so one participant may 'try harder' than another to read further down the chart when approaching their threshold limit. This introduces extra variability and thus noise to test scores that is unrelated to ocular pathology, which can significantly affect whether the endpoint is reached in a clinical trial. In addition, reading high-contrast letters at the threshold limit is not a typical everyday task and lacks real-world generalisability. Finally, there is the potential for participants to remember parts of the charts with repeated use, because typically the same left-eye and right-eye charts are used.[8] This may have significant implications for patients enrolled on multi-year or long-term follow-up in a clinical trial. Some recent research has suggested that low luminance visual acuity (LLVA) using the ETDRS chart may somewhat mitigate the first of these issues, with the implication that VA under low lighting levels may probe a slightly larger area of visual function.[9]

Choroideremia and Retinitis Pigmentosa GTPase Regulator (*RPGR*)-associated retinitis pigmentosa (*RPGR*-RP) are two X-linked inherited retinal diseases. These conditions present with nyctalopia and centripetal visual field loss.[10,11] Despite their presenting similarities, the pathogenesis and progression of choroideremia and *RPGR*-RP differ markedly. *RPGR*-RP is the most common form of X-linked RP,[11,12] caused by mutations in the *RPGR* gene that encodes for the retinitis pigmentosa GTPase regulator protein. This forms part of a protein complex involved in photoreceptor inner-to-outer segment protein trafficking.[13] Mutations inhibit this trafficking, causing primary photoreceptor degeneration. Retinal degeneration begins mid-peripherally and progresses centripetally inward and radially outward, resulting in a foveally centred, small symmetrical elliptical central island of retinal sparing and initially relatively well-preserved VA.[14] With further degeneration, the central island shrinks, and VA rapidly declines as the degeneration reaches the fovea, leading to severely impaired vision or blindness, often by the fourth decade.[12]

Choroideremia is caused by mutations in the *CHM* gene encoding for Rab Escort Protein 1 (REP1), responsible for intracellular vesicular trafficking within the retinal pigment epithelium (RPE).[15] The absence of functional REP1 causes primarily RPE degeneration followed by secondary photoreceptor degeneration.[16,17] Retinal degeneration occurs in a distinctive stellate and asymmetric pattern, with the temporal macula often relatively spared compared to the nasal macula region.[18] Once the degeneration reaches the fovea, the fixation begins to shift to the spared temporal regions. Therefore, when reading Indo-European languages (horizontally, left to right), the right eye in late-stage choroideremia will tend to read into the scotoma whereas the left eye will read into the relatively preserved visual field, with the scotoma following after the letters have been read. This may impact VA chart readings in very advanced stages of choroideremia, when degeneration encroaches on the fovea and measurement will be affected.[18,19]

A recent prospective study compared the usability of a single optotype VA test versus the ETDRS chart in patients with constricted visual fields due to inherited retinal diseases.[20] The study suggested that standard ETDRS VA testing is limited in these individuals, with VA measurement being influenced by the patient's ability to localise letters. The present retrospective study investigated how participants with inherited retinal disease, causing very constricted central retinal fields, read across the full width of the ETDRS chart by retrospective analysis of positional errors during ETDRS standard and low-luminance VA testing, and explored how the

**Key points**

- The results from this study suggest that difficulty in tracking across lines during visual acuity testing in choroideremia may cause excess errors independent of true visual acuity.
- Visual acuity assessment with single letters rather than full letter charts may be more suitable for patients with significant asymmetrical central visual field restriction such as choroideremia.
- Considering asymmetry in retinal dysfunction will aid understanding of visual function tests in such diseases and may assist identifying more suitable endpoints in clinical trials for novel therapies in choroideremia.

characteristic disease patterns may impact the validity of VA using the ETDRS chart as a clinical trial outcome measure.

## METHODS

Thirty-nine participants with confirmed pathogenic *CHM* variant mutations, 23 participants with confirmed pathogenic *RPGR* mutations and 35 controls of working age (between 18 and 65 years of age) were included in the study. Participants with inherited retinal disease were assessed as part of a screening process prior to their recruitment into gene therapy clinical trials (UK research ethical approval references: 15/LO/1379 and 16/SC/0551) at Oxford Eye Hospital. Control participants were assessed as part of the Visual Function in Inherited Retinal Disease study (ISRCTN Registration: ISRCTN24016133, UK research ethical approval reference: 20/WM/0283).[21] All data were collected in accordance with the tenets of the Declaration of Helsinki.

### Assessment of visual acuity

All participants underwent subjective refraction, using the ETDRS chart R, prior to monocular distance standard VA testing using the retro-illuminated (160 cd/m$^2$) ETDRS chart (Precision Vision; precision-vision.com/product-category/etdrs/etdrs-charts), placed at a 4-m distance in a darkened room. Participants were instructed to read down the chart, from left to right, as far as they could until they were no longer able to read any letters. Participants were encouraged to read as far as possible. The right eye was tested first using chart one, followed by the left eye using chart two.

A random sub-cohort of participants with choroideremia and controls also completed LLVA testing prior to standard VA testing. This involved the same testing procedure as used with standard VA testing outlined above, but with the addition of a 2.0 neutral density filter (Precision Vision; precision-vision.com/products/ophthalmic-supplies/examination/lenses-prisms/neutral-density-filter-for-trial-lens-frame). LLVA data were not collected for the *RPGR*-RP participants as this was not part of the operating procedures at the time of screening.

### Assessment of macular sensitivity and structure

#### Mesopic microperimetry

The Macular integrity assessment (MAIA) microperimeter (ICare CenterVue; icare-world.com/) was used to assess the central macular sensitivity surrounding the fovea (Figure 1a). A standard 10–2 test grid was used, with 4–2 bracketing threshold strategy and Goldmann size III stimulus of various intensities presented on a mesopic background (4 apostilbs ≈ 1.27 cd/m$^2$). Tests were deemed reliable if the fixation losses were ≤20%, corresponding to ≤20% of positive catch trials presented to the participant's physiological blind spot. Only choroideremia and *RPGR*-RP participants with preserved foveal function were included in this analysis. Those with completely degenerated foveal function were excluded, as it was not possible retrospectively to identify the preferred retinal location that corresponded to the VA measurement, which subsequently hindered analysis of the surrounding macular sensitivity.

Mesopic microperimetry examinations from all 39 choroideremia participants, 23 *RPGR*-RP participants and 35 controls were analysed. Eighteen participants (nine with choroideremia and nine with *RPGR*-RP) were excluded from mesopic microperimetry analyses due to >20% fixation losses, leaving a total of 30 choroideremia participants and 14 *RPGR*-RP participants. One control was excluded due to missing data, leaving a total of 34 control participants.

#### Retinal imaging

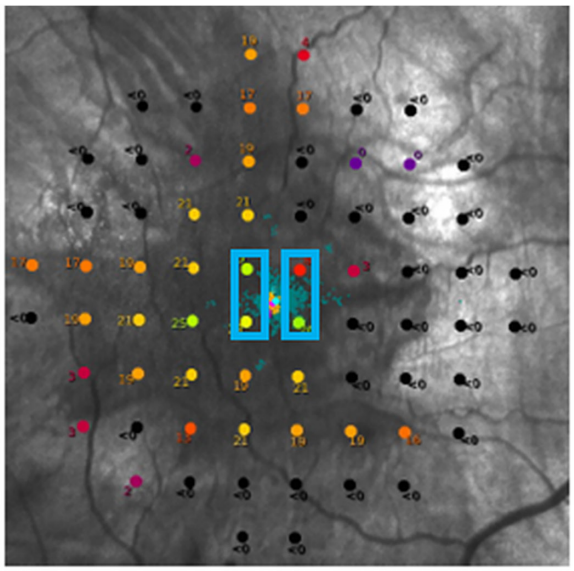
Horizontal ellipsoid zone diameters obtained via optical coherence tomography (OCT) volume scans, taken using the Heidelberg Spectralis (Heidelberg Engineering; business-lounge.heidelbergengineering.com/us/en/products/spectralis/spectralis) were used as a structural marker of central macular function in both choroideremia and *RPGR*-RP participants. A horizontal line scan through the foveal pit was selected and the OCT (Eye Explorer® software) built-in calliper was used to measure nasal and temporal ellipsoid zone length (μm) from the centre of the foveal pit, in accordance with methodology devised by Ramachandran et al.[22] (Figure 1b).

### ETDRS letter chart performance analyses

#### ETDRS column errors and weighted ETDRS column error scores

##### *ETDRS column errors*

The five Sloan letters making up each line on the ETDRS chart were categorised into assigned columns. For Chart 1 (used for right eyes), the first letter of each line was assigned the label N2, denoting two letters from the central optotype in the nasal field direction. The second letter of each line was assigned the label N1. The central letter of each line was assigned the label C. The fourth and fifth letters temporal to C on each line were assigned T1 and T2 respectively. For Chart 2 (used for the left eye), labels were a mirror of those of Chart 1, with the first letter of each line assigned the label T2 and so on, because the left eye nasal and temporal visual fields mirror those of the right eye (Figure 1c). Hence, when reading the chart line from left to right, the right eye reads



(c)

Chart 1

| Row | Snellen Acuity Equivalent | Letter column N2 N1 C T1 T2 | Number Correct at 4 meters |
|---|---|---|---|
| 1 | 20/200 | N C K Z O | 5 |
| 2 | 20/160 | R H S D K | 5 |
| 3 | 20/125 | D O V H R | 5 |
| 4 | 20/100 | C Z R H S | 5 |
| 5 | 20/80 | O N H R C | 5 |
| 6 | 20/63 | D K S N V | 5 |
| 7 | 20/50 | Z S O K N | 5 |
| 8 | 20/40 | C K D N R | 5 |
| 9 | 20/32 | S R Z K D | 2 |
| 10 | 20/25 | H Z O V S | 3 |
| 11 | 20/20 | N V D O K | 3 |
| 12 | 20/16 | V H C N O | – |
| 13 | 20/12.5 | S V H C Z | – |
| 14 | 20/10 | O Z D V K | – |
| Total column errors : | | 1 1 2 2 1 | 48 |
| Weighted error score : | | 1 | |

(b)

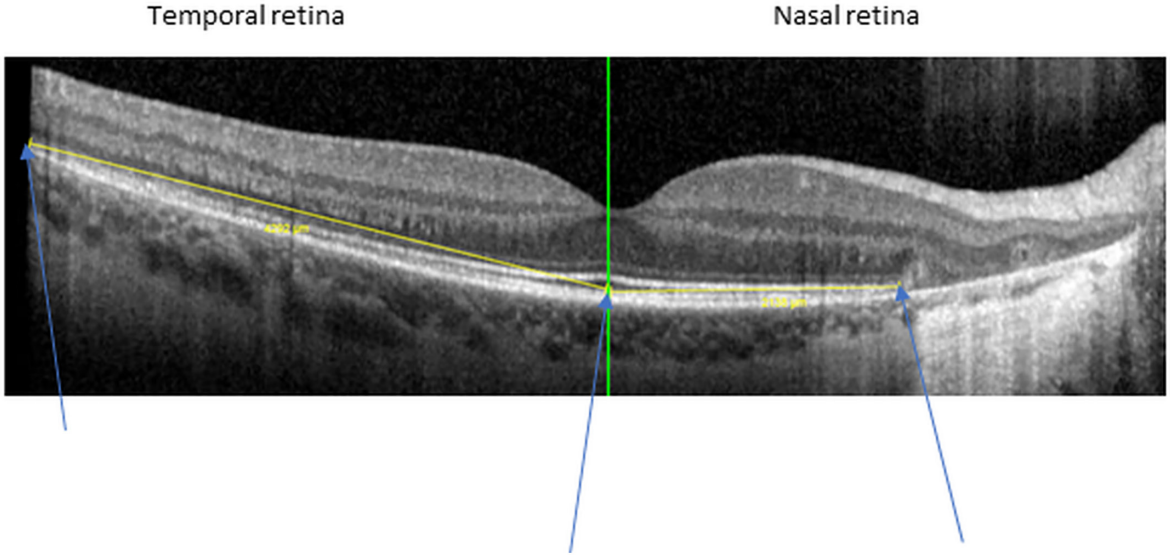



**FIGURE 1** Analysis of retinal structure and function and calculation of error scores. (a) Representative image showing the calculation of temporal-versus-nasal macular sensitivity from the difference in average sensitivity of the two points temporal and nasal to fixation measured by mesopic microperimetry. (b) Example optical coherence tomography image from a choroideremia patient. The arrows point to the foveal reference point and endpoints of ellipsoid zones. (c) Example of an Early Treatment of Diabetic Retinopathy Study (ETDRS) scoring sheet for the right eye. Columns are labelled N2, N1, C, T1 and T2 based on their position in the visual field (nasal or temporal) relative to a central position. Red circles indicate the column labels and those letters within the first column counted in the error score analysis. Total errors per column are summed below the chart, and from this, a weighted error score was calculated.

in the order N2, N1, C, T1, T2 on Chart 1, whereas the left eye reads in the order T2, T1, C, N1, N2 on Chart 2.

ETDRS letter errors in each column were defined as incorrectly read letters up to and including one row below the "threshold" row (defined as the lowest row with three or more correctly read letters), to ensure the inclusion of all correctly read letters below the assigned threshold cut-off. Any un-attempted letters within valid attempted rows were regarded as errors. Whole un-attempted rows were not counted as errors (Figure 1c). The numbers of letter errors for each assigned column were counted for both the right and left eyes for each participant to generate individual ETDRS column error scores. Total population ETDRS column error scores were

then calculated as the sum of all participant level ETDRS column error scores.

*Weighted ETDRS error scores*

In order to determine if chart reading errors were more likely to be nasal or temporal, a weighted ETDRS error score was derived for each patient. Individual participant weighted error scores were calculated from column error scores using a weighting calculation: 2(T2) + T1 – N1 – 2(N2), where T2 refers to the sum of ETDRS letter errors in column T2 and so on. This weighted formula accounted for the fact that the N2 and T2 letters subtended double the angle from the central C letter compared to N1 and T1 (C was assigned a value = 0). An overall positive weighted ETDRS error score indicated that the participant made more errors in columns on the temporal side compared to the nasal side, while a negative score indicated greater column errors on the nasal side compared to the temporal side. A weighted ETDRS error score of zero would indicate either no errors made in the attempted lines or an equal number of errors in temporal-versus-nasal columns.

## Nasal versus temporal central macular sensitivity analysis

From the microperimetry results, only the central four loci were considered because these corresponded to the anatomical region of the macula normally used for reading. The central two nasal and two temporal loci were averaged (Figure 1a) in order to provide a measure of central temporal and central nasal sensitivities. To highlight any macular sensitivity asymmetry, the nasal central macular sensitivity was subtracted from the temporal central macular sensitivity. A positive sensitivity score indicated greater sensitivity in the temporal macula, while a negative score indicated greater sensitivity in the nasal macula. Hence, a positive (greater temporal sensitivity) score on macular sensitivity would be expected to correlate with a positive (greater temporal error distribution) weighted ETDRS error score because the temporal macula feeds the nasal visual field.

## Statistical tests

Statistical analyses were performed using SPSS (version 28; IBM software; ibm.com/products/spss-statistics) and R (version 4.2.1; r-project.org). Graphical data were produced using Prism (version 9.4.1; GraphPad software; graphpad.com/features). Normality of the data was tested using the Shapiro–Wilk test. Non-parametric descriptive and statistical tests were used for non-normally distributed data. Statistical significance was set at $p \leq 0.05$. The Bonferroni correction was applied for multiple comparisons when indicated.

A cumulative link mixed model (CLMM) was employed to investigate differences in the distribution of column errors between T1 versus N1 and T2 versus N2 for each cohort of participants. Error scores were set as ordinal dependent variables, with column labels as the independent categorical variables and a post-hoc analysis of ETDRS column error scores using estimated marginal means. The CLMM is a modified version of a linear mixed model but for categorical data. This was used due to the very small range of integer numbers associated with error scores and repeated measures nature of the data (same participant assessed for each column), which meant assumptions for standard variance analyses were not met. The relationship between temporal-versus-nasal central macular sensitivity and individual participant ETDRS weighted column error scores was established using Spearman's rank correlation analysis.

# RESULTS

Standard VA was reduced significantly in both choroideremia (Kruskal–Wallis test, right and left eyes, $p < 0.001$) and *RPGR*-RP participants (Kruskal–Wallis test, right and left eyes, $p < 0.001$) when compared with controls. There was no significant difference in standard VA between choroideremia and *RPGR*-RP participants in the right (Kruskal–Wallis test, $p = 0.35$) or left eye (Kruskal–Wallis test, $p = 0.62$). For the subset of participants (23 with choroideremia and 35 controls) who completed LLVA testing, LLVA was significantly reduced in choroideremia participants compared to controls in both eyes (Mann–Whitney *U* test, right and left eyes, $p < 0.001$; Table 1).

## Comparison of the total (cohort) error scores between columns of the ETDRS chart

### Standard VA

For choroideremia participants, the right eye showed a significant stepwise increase in total column errors from N2 (23 errors) to T2 (42 errors) (CLMM, $p = 0.002$), indicating that greater errors were made on the temporal compared to the nasal side of the letter chart with the right eye (Figure 2a). Although a similar trend was also seen between N1 and T1, the difference was less marked and did not reach statistical significance. In comparison, the left eye showed no distinct trend and no significant difference in the distribution of column errors (Figure 2a and Table 2), suggesting more uniform letter error localisation.

In *RPGR*-RP participants, for both the right and left eyes, total column errors were generally consistent across columns (Figure 2b) with no statistically significant differences between error scores across the nasal versus temporal visual fields. For the right eyes, the total column errors were highest in T2 and lowest in N1 and T1 (Figure 2b) but the difference did not reach statistical significance (Table 2). For the left eyes, column errors were highest in

**TABLE 1** Participant demographics and visual acuity (VA) results for the right and left eyes.

| | Choroideremia | | *RPGR*-RP | | Controls | |
|---|---|---|---|---|---|---|
| | OD | OS | OD | OS | OD | OS |
| *Standard VA* | | | | | | |
| *n* | *39* | | *23* | | *35* | |
| Age, median (IQR) (years) | 44.9 (35.7–53.5) | | 30.8 (26.5–40.5) | | 23.8 (20.3–29.0) | |
| Standard VA, median (IQR) (ETDRS letters) | 72.0 (56.5–82.5) | 73.0 (57.0–81.5) | 65.0 (58.0–74.0) | 64.0 (59.5–73.0) | 92.0 (88.5–94.0) | 91.0 (89.0–95.0) |
| *Low luminance VA* | | | | | | |
| *n* | *23* | | N/A | | *35* | |
| Age, median (IQR) (years) | 43.9 (33.7–47.5) | | N/A | | 23.8 (20.3–29.0) | |
| Low luminance VA, median (IQR) (ETDRS letters) | 66.0 (42.5–71.5) | 67.0 (49.0–72.5) | N/A | N/A | 82.0 (80.0–84.0) | 81.0 (79.0–83.0) |

Abbreviations: ETDRS, Early Treatment of Diabetic Retinopathy study; IQR, inter-quartile range; OD, right eye; OS, left eye; RPGR-RP, X-linked retinitis pigmentosa.

T2 and lowest in C, but again, no statistically significant differences were seen (Table 2). The relatively random nature of the scores suggests that *RPGR*-RP participants did not make consistent errors in any particular column as they reached their threshold row.

In the control participants, again neither the right nor left eyes showed obvious trends in column errors (Figure 2c and Table 2). In the right eyes, errors were highest in C, T1 and T2 and lowest in N2 (Figure 2c) but these differences did not reach statistical significance. In the left eyes, total column errors were highest in N1 and lowest in N2, but again the difference was not statistically significant.

### Low luminance visual acuity

In choroideremia, the right eyes generally showed the highest LLVA total column errors in column T2 and the lowest in C. The left eyes generally showed the highest total column errors in N1 and the lowest in C (Figure 2d). Again, there were no significant differences in these distributions of letter errors in either eye (Table 2), indicating no discernible trend in letter column errors.

In the controls, the right and left eyes showed no obvious trend in error distribution (Figure 2e). Errors were lowest in N1 and highest in T2 in the right eyes. However, for the left eyes, total column errors were significantly higher in N1 (35 errors) compared to T1 (15 errors) (CLMM, $p = 0.0003$). Despite this finding, there was no statistically significant difference between the T2 and N2 columns (Table 2).

## Nasal and temporal central macular function and structure

### Mesopic central macular sensitivity

Choroideremia participants had a significantly higher two-point mean temporal-versus-nasal central macular sensitivity in the right eyes (Wilcoxon signed rank test, $p = 0.04$), consistent with the previously described sparing of the temporal macula.[18] A similar trend was seen in the left eyes, although it did not quite reach statistical significance (Table 3). In *RPGR*-RP participants, the right and left eyes had similar sensitivities across both the temporal and nasal central fields. Control participants also showed similar temporal and nasal central macular sensitivity in each eye (Table 3).

### Optical coherence tomography ellipsoid zone analyses

The horizontal foveal-centred nasal and temporal ellipsoid zones of a subset of 18 choroideremia participants and 17 *RPGR*-RP participants were measured using OCT imaging (Table 3). In participants with choroideremia, in keeping with previously published observations, the temporal ellipsoid zone was greater in extent than the nasal ellipsoid zone for the right eyes (Wilcoxon signed rank test, right eye: $p = 0.04$). The left eyes did not demonstrate any statistically significant asymmetry. Likewise, there was no evidence of asymmetrical nasal-versus-temporal ellipsoid zone extents in *RPGR*-RP participants in either the right or left eyes (Table 3).

## Correlation between weighted error scores and mesopic central macular sensitivity symmetry

### Standard visual acuity

Participant-level weighted error scores were plotted against temporal-versus-nasal central macular sensitivity differences to determine whether there was an association between the position of greatest errors on the ETDRS chart and asymmetrical macular sensitivity (Figure 3).

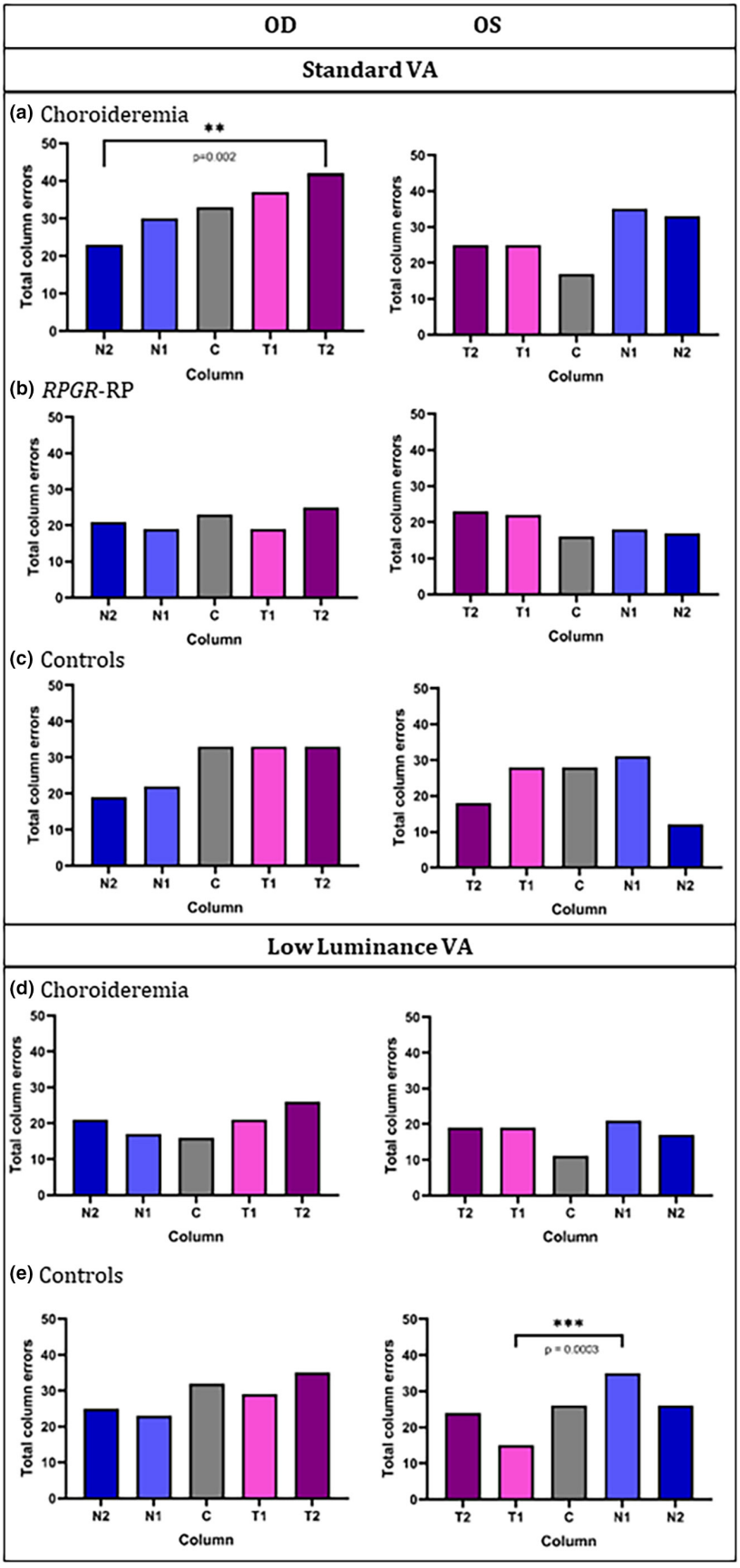



**FIGURE 2** The distributions of standard visual acuity (VA) and low luminance visual acuity (LLVA) column errors for the right (OD) and left (OS) eyes. Total column errors under standard (a–c) and low luminance (d, e) conditions were counted for choroideremia, RPGR-associated retinitis pigmentosa (RPGR-RP) and control participants. N2, N1, T2 and T1 refer to the second and first columns of letters away from the central (C) position on the nasal and temporal sides of the chart, respectively.

In choroideremia participants, the majority of right eyes yielded positive weighted error scores (indicating more errors in the temporal columns), while the majority of left eyes yielded negative weighted error scores (indicating more errors in the nasal columns) (Figure 4). Spearman's rank correlation analysis in the right eyes of choroideremia participants showed a significant positive correlation between weighted total error scores and temporal-versus-nasal central macular sensitivity ($\rho = 0.40$, $p = 0.03$; Figure 3a), consistent with the hypothesis that asymmetric central macular sensitivity causing reading into a scotoma was connected to a shift in errors towards the temporal side of the chart. However, the weighted error scores in the left eye showed no significant correlation with temporal-versus-nasal central macular sensitivity ($\rho = 0.05$, $p = 0.78$). This indicates that there was no association between central macular asymmetry and the location of column letter errors in the left eye.

In *RPGR*-RP participants, both right and left eyes yielded a slightly more even spread of positive, negative and neutral weighted error scores in each eye, indicating no definitive trend in the location of letter errors (Figure 4). Coupled with symmetrical central macular symmetry (reported above), the temporal-versus-nasal macular sensitivity showed no significant correlation with weighted total error scores in either eye (Spearman's rank; right eye: $\rho = 0.19$, $p = 0.51$; left eye: $\rho = 0.13$, $p = 0.65$; Figure 3b).

In control participants, both the right and left eyes yielded moderately positive weighted error scores, indicating more errors in the temporal columns (Figure 4). However, Spearman's rank analysis for controls (Figure 3c) again revealed no correlation between weighted total

**TABLE 2** Significance values generated by the cumulative linear mixed model analysis enabling comparison of Early Treatment of Diabetic Retinopathy Study (ETDRS) error scores in columns T2 versus N2 and T1 versus N1 for each group. N2, N1, T2 and T1 refer to the second and first columns of letters away from the central position on the nasal and temporal sides of the chart, respectively.

| Group | Eye | T2 versus N2 significance (*p*) | T1 versus N1 significance (*p*) |
|---|---|---|---|
| *Standard VA* | | | |
| Choroideremia | OD | 0.002* | 0.56 |
| | OS | 0.60 | 0.49 |
| RPGR-RP | OD | 0.93 | >0.99 |
| | OS | 0.48 | 0.75 |
| Controls | OD | 0.11 | 0.21 |
| | OS | 0.60 | 0.87 |
| *Low luminance VA* | | | |
| Choroideremia | OD | 0.71 | 0.95 |
| | OS | 0.98 | >0.99 |
| Controls | OD | 0.43 | 0.81 |
| | OS | >0.99 | 0.0003* |

Abbreviations: N, nasal; OD, Right eye; OS, left eye; RPGR-RP, X-linked retinitis pigmentosa; T, temporal; VA, Visual acuity.

*Statistical significance.

**TABLE 3** Differences in temporal-versus-nasal macular sensitivity measurements and ellipsoid zone extents in all participant groups.

| | Choroideremia | | *RPGR*-RP | | Controls | |
|---|---|---|---|---|---|---|
| | OD | OS | OD | OS | OD | OS |
| *Macular sensitivity* | | | | | | |
| *n* | *30* | | *14* | | *34* | |
| Temporal central macular sensitivity, median (IQR) | 17.8 (3.4–24.8) | 20.0 (11.0–24.8) | 14.3 (6.5–18.5) | 13.0 (8.0–18.5) | 28.0 (27.0–29.0) | 28.0 (27.0–31.0) |
| Nasal central macular sensitivity, median (IQR) | 10.5 (0.3–21.0) | 12.5 (9.0–21.8) | 12.5 (5.4–17.5) | 12.8 (11.0–19.0) | 28.0 (27.0–29.0) | 28.0 (27.0–30.0) |
| Temporal-versus-nasal central macular sensitivity, median (IQR) | 2.3 (−0.8–11.8) | 2.5 (−2.0–11.3) | 1.0 (0.0–1.9) | 0.0 (−1.0–0.0) | 0.0 (−1.0–1.0) | 0.0 (−1.0–1.5) |
| Nasal–temporal difference *p*-value, adjusted significance | 0.04* | 0.06 | 0.21 | 0.41 | 0.77 | 0.95 |
| *Ellipsoid zone analysis* | | | | | | |
| *n* | *18* | | *17* | | *N/A* | |
| Overall ellipsoid zone extent, median (IQR) (μm) | 2000 (1000–3400) | 2300 (1100–3300) | 650 (290–910) | 710 (440–990) | N/A | N/A |
| Temporal ellipsoid zone extent, median (IQR) (μm) | 1100 (740–2200) | 1100 (600–1500) | 310 (180–430) | 360 (250–580) | N/A | N/A |
| Nasal ellipsoid zone extent, median (IQR) (μm) | 500 (260–980) | 740 (200–1900) | 380 (170–550) | 370 (260–420) | N/A | N/A |
| Nasal–temporal difference *p*-value, adjusted significance | 0.04* | 0.53 | 0.33 | >0.99 | N/A | N/A |

*Note*: Statistical analysis performed using Wilcoxon signed rank test, adjusted for multiple comparisons.

Abbreviations: IQR, inter-quartile range; N/A, not applicable; OD, right eye; OS, left eye; RPGR-RP, X-linked retinitis pigmentosa.

*Significance at $p < 0.05$.

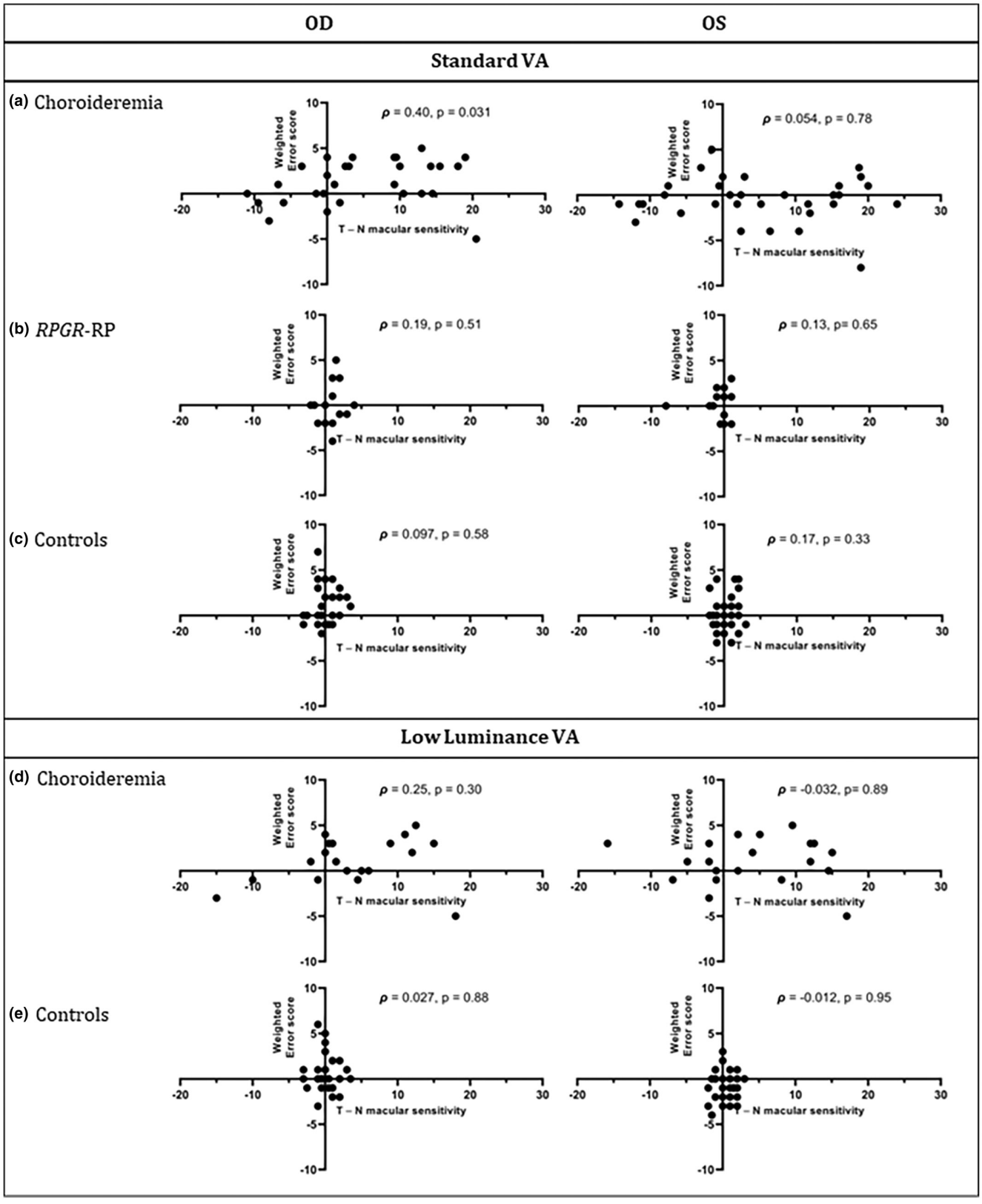



**FIGURE 3** Correlation plots and Spearman's rank analyses between two-point mean temporal-versus-nasal (T–N) central macular sensitivity and weighted error scores. Analyses were performed in (a) choroideremia, (b) RPGR-associated retinitis pigmentosa (RPGR-RP) and (c) control participants for standard visual acuity (VA), (d) choroideremia and (e) control participants for low luminance visual acuity (LLVA). OD, right eye; OS, left eye; RPGR-associated retinitis pigmentosa (RPGR-RP).

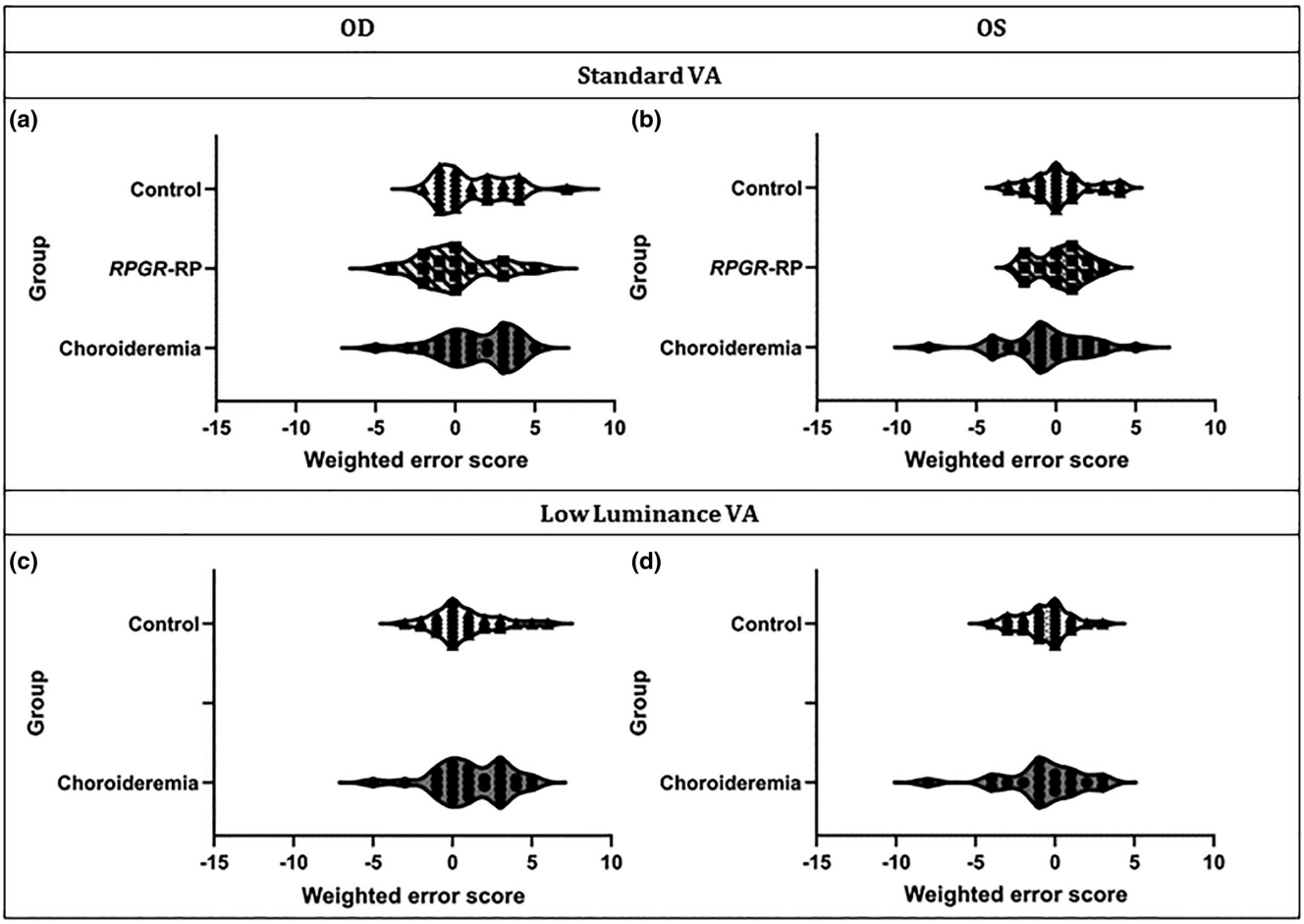



**FIGURE 4** Pirate plots of the distribution of weight error scores. Analyses were performed in choroideremia, RPGR-associated retinitis pigmentosa (RPGR-RP) and control participants in both the right and left eyes under standard (a, b) and low luminance (c, d) conditions. OD, right eye; OS, left eye; VA, visual acuity.

error scores and two-point mean temporal-versus-nasal macular sensitivity in either eye (right eye: $\rho = 0.10$, $p = 0.56$; left eye: $\rho = 0.17$, $p = 0.33$). Overall, the results suggest that symmetrical macular sensitivity tended to result in no significant trends in column error score distributions.

### Low luminance visual acuity

In the subset of 20 choroideremia participants who underwent low luminance VA testing, the majority yielded positive weighted error scores in both eyes, indicating more errors in the temporal columns (Figure 4). However, Spearman's rank analysis revealed no correlation between weighted total error scores and two-point mean temporal-versus-nasal macular sensitivity in either eye (right eye: $\rho = 0.25$, $p = 0.30$; left eye: $\rho = -0.03$, $p = 0.89$; Figure 3a); likely a consequence of the remarkably symmetrical macular sensitivity demonstrated in this group.

In the 34 control participants with microperimetry data, the majority of weighted error scores were positive in the right eye (indicating more errors in the temporal columns) and negative in the left eye (indicating more errors in the nasal columns) (Figure 4). There was no correlation identified between weighted total error scores and two-point mean temporal-versus-nasal macular sensitivity in either eye (right eye: $\rho = 0.03$, $p = 0.88$; left eye: $\rho = -0.01$, $p = 0.95$; Figure 3c); again likely a consequence of the near perfectly symmetrical macular sensitivity demonstrated in this group.

## DISCUSSION

This study investigated how asymmetric macular sensitivity loss in choroideremia affects a patient's ability to read the ETDRS chart. When the visual field scotoma lies ahead of the reading saccade (as found in the right eye), we demonstrated that such individuals make a greater proportion of total errors along the temporal (right hand) side of the ETDRS chart when reading compared to when the scotoma trails the text already read (as found in the left eye). It is possible that the position of visual field scotomas affects tracking saccades, which subsequently limits letter localisation, particularly when the patient approaches the threshold of their visual capabilities (Figure 5).[23–25] These findings correspond well with a

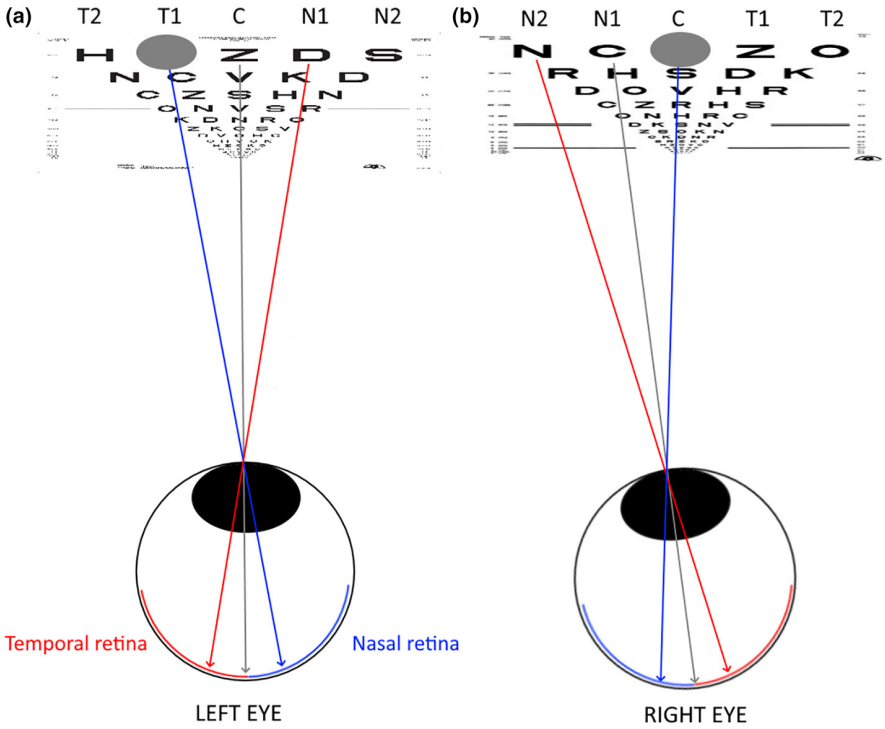



**FIGURE 5** Representative image of macular involvement during reading of the Early Treatment of Diabetic Retinopathy Study (ETDRS) chart. (a) Visual representation of the nasal (N) and temporal (T) central (C) macular field of the left eye corresponding to the temporal and nasal macula, respectively, with the eye looking straight ahead. (b) The right eye is turned inwards to simulate reading left to right, and under such circumstances subsequent letters are tracked and localised by the (comparatively more degenerated) nasal retina. The grey circles represent the temporal visual field scotoma due to nasal retinal degeneration.

previous study comparing the standard ETDRS chart to an electronic VA (EVA) chart presenting a single crowded optotype to a range of participants with rod-cone dystrophies (predominantly patients with choroideremia) and healthy controls. They found that participants read 2–3 more letters with the EVA chart when letter localisation effects were eliminated.[20]

*RPGR*-RP participants were purposely selected along with controls to compare with choroideremia participants since their central macular sensitivity typically remains symmetrical (albeit still reduced) throughout the course of the disease, and, like in choroideremia, individuals with *RPGR*-RP experience extensive visual field loss.[14] Since the *RPGR*-RP participants showed no trends in the distribution of letter chart errors, it suggests, in common with the control cohort, that they had no difficulty localising letters and reading along the letter line as they reached threshold VA. This suggests that letter chart readability and letter chart localisation are not impacted in individuals with symmetrical macular sensitivity despite extensive peripheral vision loss. However, the extent (size of residual central seeing island) of central macular sensitivity is still likely to be an influencing factor in the total number of ETDRS errors across the whole chart.[26]

Like in *RPGR*-RP, control participants showed symmetrical macular sensitivities and also demonstrated no significant trend in the distribution of letter chart errors in either eye, under standard luminance conditions. However, the left eye showed a significant increase in errors between columns N1 and T1 under low luminance conditions. This unexpected finding could be a result of fatigue effects, caused by participants tiring near threshold levels, especially as it is convention to test the left eye second. This may be overcome by randomising the order in which each eye is tested. The overall lack of trend in letter error scores in LLVA testing for choroideremia could be due to the general reduction in LLVA compared to the standard VA,[27] and therefore, not needing to read as many letters. Since this results in only the larger letter lines being read, this may precede the reading limitations caused by asymmetrical macular sensitivity and limited visual tracking. Alternatively, unlike in the right eyes in choroideremia, where there was a clear pattern of increasing error moving from the nasal to the temporal field, the fact that significant difference in errors in the control group between positions N1 and T1 was not seen between positions N2 and T2 may be explained by this being a random anomaly rather than a true difference (i.e., a Type I error).

Although this study focused on choroideremia, these findings may be applicable to other retinal diseases causing asymmetric central and paracentral macular scotoma, both inherited and acquired, such as macular telangiectasia, glaucoma and age-related macular degeneration. Difficulty in tracking across lines during ETDRS VA testing

may lead to excessive errors independent of true recognition VA. In previous clinical trials, a 15-letter VA gain (corresponding to three ETDRS chart lines) was required by the United States Food and Drug Administration (FDA) for regulatory approval of novel treatments.[28] However, in those patients who were struggling with letter localisation, they may, in fact, be achieving adequate VA increases (three lines) but were unable to achieve the 15-letter cut-off due to scotoma position and resulting letter localisation difficulties. In these patients, single optotype visual acuity testing may be more appropriate than ETDRS testing. By removing the potential confounding effects of letter localisation, patients enrolled in clinical trials may achieve the 15-letter gain required by regulatory bodies where they otherwise would not with a standard ETDRS chart.

This study had several limitations. Firstly, ETDRS standard operating testing protocols[1] required that all participants read each letter sequentially starting from the far left of the chart. However, this may not have been enforced for some participants. Those with poorer vision and small central macular seeing islands occasionally "jump" around the chart, reading letters they can localise.[1] Therefore, the distribution of errors between columns may not completely reflect the order in which they were read. Even so, the results should still reflect the letters that were seen more easily. Secondly, some of the participants (12 choroideremia and 6 *RPGR*-RP participants) had an acentric preferred retinal locus (PRL), despite having been identified as having preserved foveal function (i.e., the PRL was not equidistant from the innermost four sensitivity points; Figure 1a). This may have influenced macular sensitivity calculations if the PRL was significantly far from the central four points used for calculating macular sensitivity. It was assumed that the microperimetry-mapped PRL corresponded to the same part of the retina assessed as VA; however, this was not verified due to the lack of eye tracking during VA testing to correlate the retinal locus used. Thirdly, the threshold row in this study was defined as the lowest line with three correctly read letters. However, studies have shown that VA measurements are most accurate when subjects are encouraged to guess letters until they reach four or five incorrect letters on a single line.[29,30] Given that most letter errors tend to occur at the extremes of vision, it is possible that using a higher threshold may have provided more errors that may have given a more complete illustration of the error distribution across the ETDRS chart. Fourthly, a limitation of the testing method used is that, though participants were encouraged to guess as many letters as possible, there were no specific pre-defined cut-off criteria. In future, it would be better to have a pre-defined cut-off if using a standard ETDRS chart. This issue would also be overcome with a single-optotype, forced choice paradigm. However, given that the choroideremia and RPGR-RP participants in this study were generally young, experienced and highly motivated, we anticipate that there would be minimal effect if we were to introduce a more formal cut-off criterion. Further, it is not possible to correlate the results of this study to reading speed in day-to-day life as reading is usually performed with both eyes.

Strict age matching was not used in this study. However, all participants were of working age (between 18 and 65 years of age), and it has been shown that VA and overall visual function remain relatively constant within the working-age population.[31,32] The sample sizes for participant groups were relatively small, and not equal for either standard VA or LLVA investigations since this was a retrospective study and we included all available data. A limitation, therefore, is that this study may be underpowered to detect significant patterns in error score for the right eye. However, to the best of our knowledge, there is no similar study in the literature from which we could use data to perform a power calculation. We are, therefore, cautious when attributing a non-significant result to acceptance of the null hypothesis. A limitation intrinsic to VA testing using ETDRS charts is a lack of "forced choice"; there is some unavoidable variability regarding measurements between participants, so some participants will try harder to read letters towards the limits of their vision than others. It is difficult to standardise participant effort, despite encouragement by the examiner. This limitation could also be overcome with the use of a single crowded optotype VA test that uses a forced choice paradigm, such as the Emmes Corporation Electronic Visual Acuity (EVA) charts,[20] and, as discussed above, this eliminates the requirement of the patient to track across a line, thereby removing the issue of letter localisation.

## CONCLUSION

Despite the popularity of the ETDRS letter chart, research into its suitability as a "one-size-fits-all" approach for all causes of visual impairment is sparse. To the best of our knowledge, this is the first study to investigate directly the impact of central macular sensitivity loss on the ability to read along the ETDRS letter chart in inherited retinal disease. The results of this study suggest that the ETDRS chart may be less suited for conditions with significant asymmetric central macula retinal sensitivities and large central scotomas since letter localisation becomes an additional source of variability. Future clinical trials requiring accurate VA assessment, for conditions with either central macula sensitivity asymmetry or large central scotomas such as geographic atrophy, may be better suited using VA tests with single crowded optotypes.

### AUTHOR CONTRIBUTIONS

**Kwame A. Baffour-Awuah:** Conceptualization (equal); data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); writing – original draft (equal); writing – review and editing (equal). **Laura J. Taylor:** Conceptualization (equal); data curation (equal);

formal analysis (equal); investigation (equal); methodology (equal); writing – original draft (equal); writing – review and editing (equal). **Amandeep S. Josan:** Conceptualization (equal); data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); writing – original draft (equal); writing – review and editing (equal). **Jasleen K. Jolly:** Conceptualization (equal); data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); writing – original draft (equal); writing – review and editing (equal). **Robert E. MacLaren:** Conceptualization (equal); data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); writing – original draft (equal); writing – review and editing (equal).

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## CONFLICT OF INTEREST STATEMENT

The authors report no conflicts of interest and have no proprietary interest in any of the materials mentioned in this article.

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## REFERENCES

1. Ferris FL 3rd, Kassoff A, Bresnick GH, Bailey I. New visual acuity charts for clinical research. *Am J Ophthalmol*. 1982;94:91–6.
2. Bailey IL, Lovie JE. New design principles for visual acuity letter charts. *Am J Optom Physiol Opt*. 1976;53:740–5.
3. Shamir RR, Friedman Y, Joskowicz L, Mimouni M, Blumenthal EZ. Comparison of Snellen and Early Treatment Diabetic Retinopathy Study charts using a computer simulation. *Int J Ophthalmol*. 2016;9:119–23.
4. Holladay JT. Proper method for calculating average visual acuity. *J Refract Surg*. 1997;13:388–91.
5. Beck RW, Maguire MG, Bressler NM, Glassman AR, Lindblad AS, Ferris FL. Visual acuity as an outcome measure in clinical trials of retinal diseases. *Ophthalmology*. 2007;114:1804–9.
6. Kaiser PK. Prospective evaluation of visual acuity assessment: a comparison of Snellen versus ETDRS charts in clinical practice (an AOS Thesis). *Trans Am Ophthalmol Soc*. 2009;107:311–24.
7. Schwartz SH. Visual perception: a clinical orientation. New York, New York: McGraw-Hill; 1999.
8. Bokinni Y, Shah N, Maguire O, Laidlaw DA. Performance of a computerised visual acuity measurement device in subjects with age-related macular degeneration: comparison with gold standard ETDRS chart measurements. *Eye*. 2015;29:1085–91.
9. Wood LJ, Jolly JK, Josan AS, Buckley TMW, MacLaren RE. Low luminance visual acuity and low luminance deficit in choroideremia and RPGR-associated retinitis pigmentosa. *Transl Vis Sci Technol*. 2021;10:28. https://doi.org/10.1167/tvst.10.2.28
10. Mitsios A, Dubis AM, Moosajee M. Choroideremia: from genetic and clinical phenotyping to gene therapy and future treatments. *Ther Adv Ophthalmol*. 2018;10:2515841418817490. https://doi.org/10.1177/2515841418817490
11. Talib M, van Schooneveld MJ, Thiadens AA, Fiocco M, Wijnholds J, Florijn RJ, et al. Clinical and genetic characteristics of male patients with RPGR-associated retinal dystrophies: a long-term follow-up study. *Retina*. 2019;39:1186–99.
12. Cehajic Kapetanovic J, McClements ME, Martinez-Fernandez de la Camara C, MacLaren RE. Molecular strategies for RPGR gene therapy. *Genes*. 2019;10:674. https://doi.org/10.3390/genes10090674
13. Khanna H. Photoreceptor sensory cilium: traversing the ciliary gate. *Cells*. 2015;4:674–86.
14. Milam AH, De Castro EB, Smith JE, Tang WX, John SK, Gorin MB, et al. Concentric retinitis pigmentosa: clinicopathologic correlations. *Exp Eye Res*. 2001;73:493–508.
15. Khan KN, Islam F, Moore AT, Michaelides M. Clinical and genetic features of choroideremia in childhood. *Ophthalmology*. 2016;123:2158–65.
16. Roberts MF, Fishman GA, Roberts DK, Heckenlively JR, Weleber RG, Anderson RJ, et al. Retrospective, longitudinal, and cross sectional study of visual acuity impairment in choroideraemia. *Br J Ophthalmol*. 2002;86:658–62.
17. Aleman TS, Han G, Serrano LW, Fuerst NM, Charlson ES, Pearson DJ, et al. Natural history of the central structural abnormalities in choroideremia: a prospective cross-sectional study. *Ophthalmology*. 2017;124:359–73.
18. Jolly JK, Xue K, Edwards TL, Groppe M, MacLaren RE. Characterizing the natural history of visual function in choroideremia using microperimetry and multimodal retinal imaging. *Invest Ophthalmol Vis Sci*. 2017;58:5575–83.
19. Sadda SR, Wilkinson C, Hinton D, Wiedemann P, Freund KB, Sarraf D, editors. Ryan's retina. 7th ed. Amsterdam, the Netherlands: Elsevier; 2022.
20. Jolly JK, Juenemann K, Boagey H, Nadsady M, Bridge H, Maclaren RE. Validation of electronic visual acuity (EVA) measurement against standardised ETDRS charts in patients with visual field loss from inherited retinal degenerations. *Br J Ophthalmol*. 2020;104:924–31.
21. Taylor LJ, Josan AS, Stratton I, Jolly JK, MacLaren RE. A cross-sectional study to assess the clinical utility of modern visual function assessments in patients with inherited retinal disease: a mixed methods observational study protocol. *BMC Ophthalmol*. 2023;23:234. https://doi.org/10.1186/s12886-023-02974-6
22. Ramachandran R, Cai C, Lee D, Epstein B, Locke KG, Birch D, et al. Reliability of a manual procedure for marking the EZ endpoint location in patients with retinitis pigmentosa. *Transl Vis Sci Technol*. 2016;5:6. https://doi.org/10.1167/tvst.5.3.6
23. McDonald MA, Stevenson CH, Kersten HM, Danesh-Meyer HV. Eye movement abnormalities in glaucoma patients: a review. *Eye Brain*. 2022;14:83–114.
24. Van der Stigchel S, Bethlehem RA, Klein BP, Berendschot TT, Nijboer TC, Dumoulin SO. Macular degeneration affects eye movement behavior during visual search. *Front Psychol*. 2013;4:579. https://doi.org/10.3389/fpsyg.2013.00579
25. Bullimore MA, Bailey IL. Reading and eye movements in age-related maculopathy. *Optom Vis Sci*. 1995;72:125–38.
26. Jolly JK, Couldridge-Smith CE, Xue K, MacLaren RE. The impact of progressive visual field constriction on reading ability in an inherited retinal degeneration. *Ophthalmologica*. 2020;243:207–16.
27. Wood LJ, Jolly JK, Buckley TM, Josan AS, MacLaren RE. Low luminance visual acuity as a clinical measure and clinical trial outcome measure: a scoping review. *Ophthalmic Physiol Opt*. 2021;41:213–23.
28. Joussen AM, Lehmacher W, Hilgers RD, Kirchhof B. Is significant relevant? Validity and patient benefit of randomized controlled clinical trials on age-related macular degeneration. *Surv Ophthalmol*. 2007;52:266–78.

29. Shah N, Dakin SC, Whitaker HL, Anderson RS. Effect of scoring and termination rules on test–retest variability of a novel high-pass letter acuity chart. *Invest Ophthalmol Vis Sci*. 2014;55:1386–92.
30. Carkeet A. Modeling logMAR visual acuity scores: effects of termination rules and alternative forced-choice options. *Optom Vis Sci*. 2001;78:529–38.
31. Pitts DG. Visual acuity as a function of age. *J Am Optom Assoc*. 1982;53:117–24.
32. Klein R, Klein BE, Linton KL, De Mets DL. The Beaver Dam Eye Study: visual acuity. *Ophthalmology*. 1991;98:1310–5.

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