Bilateral Symmetry of Visual Function Loss in Cone–Rod Dystrophies

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PURPOSE. To investigate bilateral symmetry of visual impairment in cone–rod dystrophy (CRD) patients and understand the feasibility of clinical trial designs treating one eye and using the untreated eye as an internal control.

METHODS. This was a retrospective study of visual function loss measures in 436 CRD patients followed at the Ophthalmology Department of the Catholic University in Rome. Clinical measures considered were best-corrected visual acuity, focal macular cone electoretinogram (fERG), and Ganzfeld cone-mediated and rod-mediated electoretinograms. Interocular agreement in each of these clinical indexes was assessed by t- and Wilcoxon tests for paired samples, structural (Deming) regression analysis, and intraclass correlation. Baseline and follow-up measures were analyzed. A separate analysis was performed on the subset of 61 CRD patients carrying likely disease-causing mutations in the ABCA4 gene.

RESULTS. Statistical tests show a very high degree of bilateral symmetry in the extent and progression of visual impairment in the fellow eyes of CRD patients.

CONCLUSIONS. These data contribute to a better understanding of CRDs and support the feasibility of clinical trial designs involving unilateral eye treatment with the use of fellow eye as internal control.

Keywords: cone dystrophy, interocular, visual acuity, visual function, electroretinography

One–rod dystrophies (CRDs) are a family of inherited diseases characterized by the progressive loss of the retina photoreceptors, with a primary loss of cones, typically followed by loss of rods.1–7

Characteristically CRDs lead to early impairment of vision, being a major cause of severe visual impairment and blindness in children and young adults.8 There is presently no cure for CRDs, but a number of promising therapeutic strategies are under investigation at the preclinical level.9–11

In the great majority of cases CRDs affect both eyes, and it is the general clinical impression that the loss of visual function is symmetrical in the two eyes of CRD patients.12,13 This view is supported by quantitative studies, reporting bilateral symmetry in fundus appearance,14–16 visual acuity,17 and multifocal ERG recordings.18 These studies, however, are limited to small patient cohorts.

Quantifying bilateral symmetry of visual loss in a large cohort of CRD patients may contribute to a better knowledge of these diseases and help design clinical trials, because bilateral symmetry is a necessary condition for trial designs with unilateral treatment and the use of fellow eye as a control, as is typically the case for retinal gene therapy.18

The present retrospective study examines bilateral symmetry in the loss of visual function in 436 typical CRD patients, analyzing best-corrected visual acuity, Ganzfeld cone-mediated electoretinogram (cone ERG), Ganzfeld rod-mediated electoretinogram (rod ERG), and focal macular electoretinogram (fERG) data. The analysis addresses the symmetry of baseline values and visual decay over time in the fellow eyes. A dedicated analysis has been performed for the subset of 61 CRD patients with likely disease-causing mutations in the ABCA4 gene.

Using a set of complementary statistical tests to overcome individual test limitations, we found that visual impairment in CRDs displays a high degree of bilateral symmetry.

PATIENTS AND METHODS

This retrospective study analyzed clinical data from CRD Caucasian patients clinically followed at the Visual Epidemiology unit of the Università Cattolica del Sacro Cuore in Rome in the years 1999 to 2015. As a comparison, data from 40 normal control subjects are also illustrated.
Patients had a diagnosis of CRD based on history, clinical findings, and ERG abnormalities. They had sought consultation because of visual symptoms or as relatives of affected patients. After the first visit, patients were invited to adhere to the institutional schedule of at least one visit per year, with the exact scheduling established by an independent administrative office. All patients gave informed consent to participate to the study. The study adhered to the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board.

Study patients met the following inclusion criteria: typical CRD with a cone–rod pattern of retinal dysfunction, as determined by International Society for Clinical Electrophysiology of Vision (ISCEV) standard Ganzfeld electrotetrogram–mediated ERG was recorded in response to white 50-cd/m² mediated ERG was recorded in response to the 41-Hz sinusoidal 95% luminance modulation of the central red field. For each recording, fERG signals were amplified (100,000-fold), band-pass filtered (1–100 Hz; 6 dB/Oct), and averaged (12-bit resolution, 2-kHz sampling rate, 1200–1600 repetitions in six to eight blocks). Offline discrete Fourier analysis quantified the amplitude of the response first harmonic at 41 Hz.

**Data Acquisition and Analysis**

All measures were obtained monocularly on each eye in each patient. The usual routine testing sequence was as follows: right–left–left–right. Attributed to each eye was the average value of its two recordings.

Visual acuity and fERG data derive from visits performed between 1999 and 2015 under the same test and recording conditions. Ganzfeld cone-mediated ERG and Ganzfeld rod-mediated ERG data derive from visits performed between 2006 and 2015 under the same test and recording conditions. All ERG data analyzed in this study derive from measures performed with eyelid electrodes.

In agreement with previous studies, quantitative analyses were performed on the logarithm of the minimal angle of resolution (logMAR) for visual acuity fractions to minimize nonnormality, the b-wave amplitude for Ganzfeld ERGs, and the first harmonic amplitude for the macular fERG.

Baseline data (i.e., the measures obtained on the first visit of the patient recorded in the database) were available for all four measures. Data documenting disease progression were available only for fERG and visual acuity.

The similarity of disease progression in the two eyes was studied comparing intraocular decays in fERG and logMAR values in the two eyes after 1 to 4 years from baseline. To this purpose, we quantified logMAR and fERG variations from baseline binning data in two time windows. These windows spanned from 1 to 2.5 years from baseline and from 2.5 to 4 years from baseline, respectively. In either time bin only one entry was considered for each patient. Whenever more than one entry was available for a patient, the entry at the time closer to the center of the time bin was selected.

**Statistical Analysis**

Because the study aimed at quantifying the extent to which the left and right eye of CRD patients were similarly affected by the disease, statistical analyses were performed considering both baseline values and intraocular decay values.

For each analyzed measure (basal value and decay value) we performed a battery of tests to compare the two eyes. Specifically, we performed tests for paired data (t- and Wilcoxon rank tests) to assess whether the mean/median of the difference between left and right eye was significantly different from zero. The Deming regression was used for testing the agreement between left and right eye and the significance of a constant and a proportional bias. If the left and right eye values are plotted on an x-y graph, the slope and the intercept of a straight line fitted to the data points reveals the nature and magnitude of any bias present. If no bias is present, the estimated slope will not be significantly different from zero, and the regression line will correspond to the identity line. For computing the standard error and the nonparametric 95%
confidence interval of the slope and intercept, 5000 iterations of bootstrap resampling were used.23

For quantifying the degree of agreement between the measures obtained in the two eyes, the intraclass correlation coefficient (ICC) was also computed24 by using a two-way mixed model with measures of absolute agreement. According to previous studies, ICC data can be interpreted as follows: ICCs between 0.5 and 0.6 indicate moderate agreement; 0.7 to 0.8 indicates strong agreement; and >0.8 indicates almost perfect agreement (http://statstodo.com [in the public domain]). Alternatively, ICC < 0.4 = poor; 0.40–0.75 = good; > 0.75 = excellent.25

As a visual reference we also plotted the Bland-Altman graphs.

Matched-paired tests, ICC, and linear regression were performed using SPSS for Windows v. 20 (IBM Corp., Armonk, NY, USA). Deming regression was performed using MethComp package v. 1.22.2 of R v. 2.13.0 (R Foundation for Statistical Computing, Vienna, Austria). Additional controls were performed using Prism v.6.0 (GraphPad Software, Inc., La Jolla, CA, USA) and JMP v11.0.0 (SAS Institute, Cary, NC, USA). Data plotting was performed using Origin v. 8.5 (OriginLab Corporation, Northampton, MA, USA). A 2-tailed significance level was set at \( z = 0.05 \).

RESULTS

The CRD patient cohort we studied comprised 436 individuals, 208 males and 228 females, with an average age at first visit (baseline) of 31.21 ± 18.85 years (age range, 4–83 years); age stratification at baseline, 60 patients (<10 years), 92 (11–20 years), 61 (21–30 years), 63 (31–40 years), 72 (41–50 years), 42 (51–60 years), and 33 (61–83 years). We analyzed measures from four clinical tests. These included best-corrected visual acuity values (converted to log decimal values of the logMAR) and the response amplitudes of three electroretinographic tests (Ganzfeld rod ERG, Ganzfeld cone ERG, and fERG recorded in response to the stimulation of the central macular 18\(^\circ\)). To avoid confusion between the actual measurement values and the clinical tests from which the measure derives, the latter will be referred to as clinical indexes.

Intereye Comparison of Baseline Values

Table 1 summarizes for each clinical index the average baseline values and number of patients for whom we had bilateral data in the CRD cohort. For a comparison, electroretinographic data from 40 control subjects are also illustrated (average age, 33.6 ± 8.55 years; age range, 20–50 years).

The results of the statistical analyses testing bilateral symmetry in baseline measurements are briefly reported in the figures and shown in detail in Table 2.

For all four clinical indexes these results can be thus summarized: (1) The mean/median of the interocular difference is not significantly different from zero; (2) Deming regression analysis best linear fit is not significantly different from zero.
from the line indicating identity of the two eye measurements; and (3) the ICC has values above 0.8, indicating a very good to excellent quantitative agreement between paired measurements.25,26

The same results are obtained if this analysis is limited to the subset of patients in a more compact age range (20–50 years; see Supplementary Tables S1 and S2).

Taken together, these results indicate a very high degree of bilateral symmetry in baseline measurements of best-corrected visual acuity, rod ERG, cone ERG, and fERG in CRD patients.

Comparison of Disease Progression in the Fellow Eyes

We next tested whether disease progression was the same in the left and right eye of CRD patients.

This analysis comprised two parts. Firstly, we repeated the analysis of baseline measurements applying a lower threshold cutoff, to focus on patients for whom a decay in the specific clinical index would still be detectable. Secondly, we analyzed
the degree of similarity of visual function decay over time between the fellow eyes.

Follow-up data were available for logMAR and fERG measurements. In agreement with previous studies we set a bottom threshold value of $-0.69$ for logMAR (corresponding to 2/10, the penultimate line in the Snellen chart) and $0.5 \mu V$ for fERG (a value previously identified as a cutoff to avoid floor effects in fERG time lines$^{21}$). Bilateral measures above the set thresholds were available from 195 patients for fERG and 184 patients for logMAR (Table 1).

Figures 5 and 6 illustrate baseline data above cutoff for logMAR and fERG, respectively.

Summary statistics for intereye statistical tests are detailed in Table 3 and reported schematically in Figures 4 and 5. In summary, all tests indicated a high degree of similarity between the fellow eye baseline measurements, also in the dataset restricted to patients with measures above cutoff.

To test the degree of similarity between the fellow eyes in visual function decay over time we focused on measures obtained from 1 to 4 years from baseline, subdividing this interval in two equal bins, spanning from 1 to 2.5 years and
**Table 2.** Baseline Statistics of Intereye Differences in Clinical Indexes for CRD Patients

<table>
<thead>
<tr>
<th></th>
<th>Rod ERG, μV</th>
<th>Cone ERG, μV</th>
<th>fERG, μV</th>
<th>logMAR</th>
<th>logMAR &gt; −0.69</th>
<th>fERG &gt; 0.5 μV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>N</td>
<td>Paired t Wilcoxon</td>
<td>N</td>
<td>Paired t Wilcoxon</td>
<td>N</td>
</tr>
<tr>
<td>Matched paired tests</td>
<td>Probability</td>
<td>98</td>
<td>0.1523</td>
<td>0.7775</td>
<td>0.0000</td>
<td>128</td>
</tr>
<tr>
<td></td>
<td>Estimate</td>
<td>95%CI low</td>
<td>95%CI up</td>
<td>95%CI low</td>
<td>95%CI up</td>
<td>95%CI low</td>
</tr>
<tr>
<td>Deming regression</td>
<td>Intercept</td>
<td>−1.770</td>
<td>−4.650</td>
<td>1.116</td>
<td>−0.939</td>
<td>−2.928</td>
</tr>
<tr>
<td></td>
<td>Slope</td>
<td>1.071</td>
<td>1.000</td>
<td>1.114</td>
<td>1.118</td>
<td>0.984</td>
</tr>
<tr>
<td></td>
<td>ICC</td>
<td>0.948</td>
<td>0.923</td>
<td>0.965</td>
<td>0.834</td>
<td>0.772</td>
</tr>
</tbody>
</table>

CI, confidence interval of the mean; low, lower bound; up, upper bound.

**Table 3.** Summary of Statistical Tests of Bilateral Symmetry on logMAR and fERG Decay With Time in CRD Patients

<table>
<thead>
<tr>
<th></th>
<th>T1, 1–2.5 Years</th>
<th>T2, 2.5–4 Years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Delta logMAR</td>
<td>Delta fERG</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>Mean</td>
</tr>
<tr>
<td>OD</td>
<td>48</td>
<td>0.1408</td>
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<tr>
<td>OS</td>
<td>127.5</td>
<td>0.2743</td>
</tr>
<tr>
<td>OD-OS</td>
<td>30.3</td>
<td>0.01302</td>
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</table>

Matched paired tests

<table>
<thead>
<tr>
<th></th>
<th>Paired t Wilcoxon</th>
<th>Paired t Wilcoxon</th>
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</thead>
<tbody>
<tr>
<td>Probability</td>
<td>0.61</td>
<td>0.81</td>
</tr>
</tbody>
</table>

Deming regression

<table>
<thead>
<tr>
<th></th>
<th>Estimate</th>
<th>95%CI low</th>
<th>95%CI up</th>
<th>Estimate</th>
<th>95%CI low</th>
<th>95%CI up</th>
<th>Estimate</th>
<th>95%CI low</th>
<th>95%CI up</th>
<th>Estimate</th>
<th>95%CI low</th>
<th>95%CI up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>−0.005</td>
<td>−0.073</td>
<td>0.064</td>
<td>0.008</td>
<td>−0.163</td>
<td>0.180</td>
<td>−0.045</td>
<td>−0.038</td>
<td>0.111</td>
<td>0.004</td>
<td>−0.202</td>
<td>0.193</td>
</tr>
<tr>
<td>Slope</td>
<td>0.943</td>
<td>0.7255</td>
<td>1.161</td>
<td>0.992</td>
<td>0.722</td>
<td>1.263</td>
<td>0.801</td>
<td>0.58</td>
<td>1.024</td>
<td>1.019</td>
<td>0.719</td>
<td>1.319</td>
</tr>
<tr>
<td>ICC</td>
<td>0.789</td>
<td>0.652</td>
<td>0.876</td>
<td>0.682</td>
<td>0.525</td>
<td>0.794</td>
<td>0.764</td>
<td>0.623</td>
<td>0.858</td>
<td>0.676</td>
<td>0.510</td>
<td>0.796</td>
</tr>
</tbody>
</table>
from 2.5 to 4 years, respectively. Follow-up data in the 1- to 4-year interval from baseline were available for 48 patients for logMAR and 64 patients for fERG. For each time bin, only one datum per patient was considered (see Methods).

Figure 7A plots the left eye logMAR decay versus the corresponding right eye decay after 1 to 2.5 years from baseline (left) and after 2.5 to 4 years from baseline (right). Figure 7B similarly illustrates the available measures of fERG decline from baseline. Preliminary tests showed high intereye concordance between baseline values in this subset, as well as independence of decline values from baseline (not shown).

The results of the statistical tests assessing the similarity of the fellow eyes in visual function decay over time are shown in Table 3 and reported schematically in Figure 7.

As for baseline data, all tests indicate a high degree of similarity in disease progression between the fellow eyes of CRD patients as measured by logMAR and fERG decay from baseline.

**Bilateral Symmetry in ABCA Mutation Carriers**

In light of the increasing interest focused on CRDs associated with \( ABCA4 \) gene mutations, we studied the degree of bilateral symmetry in the subset of the 61 CRD patients in our cohort who carried likely disease-causing mutations in the \( ABCA4 \) gene.

Among these patients, 56 had bilateral logMAR measurements and 60 had bilateral fERG measurements. Only baseline measurements were available. These data are shown in Figure 8.

The results of the tests assessing the similarity of visual function in the fellow eyes are shown in Table 4 and reported schematically in Figure 8. They indicate a high degree of
similarity in visual function loss in the fellow eyes of ABCA4 CRD patients as revealed by logMAR and fERG measurements. Rating ABCA4 mutation in three classes of increasing severity according to Fujinami et al., these patients comprised 12 (20%) class 1, 32 (53%) class 2, and 16 (27%) class 3 cases. No significant effect of mutation severity on intereye differences was observed in the sample. This analysis may suffer, however, from the limited number of cases included.

**DISCUSSION**

We analyzed interocular variability in visual acuity, fERG, full-field rod-mediated ERG, and full-field cone-mediated ERG in CRDs, using data from 436 CRD patients. The degree of quantitative similarity between the measurements obtained in the two eyes of each patient was assessed using three different and complementary statistical tests, and results were considered in the context of intereye variability in a cohort of normal control patients.

The statistical tests used addressed whether the mean/median intereye difference differed from zero (paired tests), determined whether intereye data agreed without constant or proportional biases (Deming regression), and quantified the degree of quantitative agreement between the measures obtained in the two eyes (ICC). These tests complemented each other and together provided a more complete view than a single test would have done.

Our results show that interocular differences in visual acuity, fERG, and full-field cone- and rod-mediated ERGs in CRD patients are not statistically significant; left and right eye measurements display high values of interclass correlation; and their relationship can be fitted by an equality line. Interocular variability in CRD patients was very similar to that of normal control subjects.

This analysis was prompted by and parallels a similar study performed for retinitis pigmentosa patients. Our results formalize and quantify, on a large patient cohort, the general clinical impression that the loss of visual function is commonly symmetrical in the two eyes of CRD patients. This study complements previous observations, based on a limited number of patients, reporting bilateral symmetry of the fellow eyes in CRD fundus appearance, visual acuity, and multifocal ERG recordings.

The main novel features of the present study are the high amount of patient data and the use of a set of complementary tests to assess the relatedness of quantitative traits between the fellow eyes for the four clinical indexes considered.

The present analysis has been focused on visual impairments as assessed with functional measures only (visual acuity, TABLE 4. Summary of Statistical Tests of Bilateral Symmetry on Baseline Measurements of the ABCA4 Mutation Carrier

<table>
<thead>
<tr>
<th>ABCA4</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>OD</td>
<td>56</td>
<td>-0.855</td>
<td>0.496</td>
<td>60</td>
<td>0.504</td>
<td>0.401</td>
</tr>
<tr>
<td>OS</td>
<td>-0.809</td>
<td>0.556</td>
<td>0.408</td>
<td>-0.029</td>
<td>0.248</td>
<td></td>
</tr>
<tr>
<td>OD-OS</td>
<td>-0.046</td>
<td>0.408</td>
<td>0.408</td>
<td>-0.029</td>
<td>0.248</td>
<td></td>
</tr>
<tr>
<td>Paired t Wilcoxon</td>
<td>0.4</td>
<td>0.267</td>
<td>0.376</td>
<td>0.524</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deming regression</td>
<td>0.196</td>
<td>-0.099</td>
<td>0.491</td>
<td>-0.085</td>
<td>-0.189</td>
<td>0.020</td>
</tr>
<tr>
<td>ICC</td>
<td>0.701</td>
<td>0.539</td>
<td>0.813</td>
<td>0.825</td>
<td>0.724</td>
<td>0.892</td>
</tr>
</tbody>
</table>

Abbreviations as in Tables 1 and 2.
fERG, and Ganzfeld cone and rod electroretinograms). A necessary future step will be to understand the degree of bilateral symmetry assessed through quantitative examinations of the retinal structure. This is particularly important considering that while functional analysis can powerfully assess visual function, structural examinations are the elective tools to determine the characteristics and pattern of photoreceptor loss at the basis of the disease.

The finding of a high degree of bilateral symmetry of the fellow eyes in functional visual impairment in CRDs may contribute to a better understanding of the natural course of these pathologies. The present analysis showed that bilateral symmetry characterized both the extent and the rate of functional visual loss in CRD patients. This suggests a high degree of interocular congruence in the pattern and natural history of functional retinal degeneration. Why this should be the case is an open question.

A number of studies report considerable symmetry in the central retinal features of the fellow eyes in normal subjects, including central cone photoreceptor density evaluated with adaptive optics, optical coherence tomography measures of macular thickness, macular pigment density, and macular blood flow. In light of these data, one could speculate that under the action of common mechanistic noxious determinants (such as genetic mutations, environmental and systemic factors), the likelihood of photoreceptor dysfunction and loss would have a similar retinal distribution in the two eyes of a patient, eventually resulting in bilateral symmetry of functional visual loss.

The high degree of bilateral symmetry in functional visual loss in CRDs seems to support the feasibility of trial designs treating one eye and employing the fellow eye as internal control.

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