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RESEARCH ARTICLE

Minimum Ganglion Cell Layer Thickness is the Best Structural Predictor of Visual Function in Leber Hereditary Optic Neuropathy

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ABSTRACT

Background: Patients with Leber hereditary optic neuropathy, a genetic cause of severe optic atrophy and blindness, demonstrate characteristic structural changes measurable through optical coherence tomography, including initial swelling of the retinal nerve fiber layer and progressive thinning of the ganglion cell layer. After symptomatic conversion, patients experience progressive vision loss, often with dense central scotomas.

Aims: This study aims to explore the predictive potential of ocular structural measurements to visual function in patients with Leber hereditary optic neuropathy.

Methods: Medical records were reviewed retrospectively for patients with Leber hereditary optic neuropathy clinical testing. Structural measurements, including the average thickness of the ganglion cell layer, the retinal nerve fiber layer, and minimum thickness of the ganglion cell layer, measured through optical coherence tomography were obtained along with measures of visual function such as visual acuity and mean deviation of visual field testing. Simple and multivariable linear regressions were performed to determine correlations between structural measurements and visual functions. Analyses were conducted for all patients, and subgroup of symptomatic patients.

Results: Overall, 92 eyes were included with 78 symptomatic eyes. Across all patients, the minimum ganglion cell layer thickness had the highest correlation coefficient with visual acuity (Beta -0.632, adjusted R^2 0.396) and with visual field function (Beta 0.572, adj. R^2 0.320) compared to the average thickness of the ganglion cell layer (Beta 0.474, adj. R^2 0.216) and retinal nerve fiber layer (Beta 0.481, adj. R^2 0.223). In multivariate analysis, the minimum ganglion cell layer thickness was the only significant measurement that correlated with visual acuity across all eyes (Beta -0.527, $P<0.001$) and symptomatic eyes (Beta -0.479, $P<0.001$). The minimum ganglion cell layer thickness (Beta 0.440, $P<0.001$) and retinal nerve fiber layer average thickness (0.258, $P=0.023$) were significant structural measurements correlating to visual field function.

Conclusion: The minimum ganglion cell layer thickness is the best structural measure predictor for visual field and visual acuity compared to other common structural measurements in patients with Leber hereditary optic neuropathy. It is a good predictor even when evaluating only symptomatic eyes. Additional attention toward the minimum ganglion cell layer thickness may offer further insight into a patient's visual potential and the natural history of this disease.

INTRODUCTION

Leber hereditary optic neuropathy (LHON) is a rare maternally inherited mitochondrial disease characterized by profound progressive bilateral central vision loss.[1] More than 95% of LHON cases are accounted for by three pathogenic point mutations of mitochondrial DNA: G11778A, T14484C, and G3460A.[2] Incomplete penetrance of the disease suggests additional genetic or environmental factors are involved in the conversion from the asymptomatic carriers to the symptomatic affected stages.[3] The deterioration of visual acuity typically progresses over a period of days to weeks, with involvement of the other eye occurring days to months after.[1,4]

On clinical examination, peripapillary microangiopathy can be observed in both symptomatic and asymptomatic LHON patients.[1,5–7] After conversion, the pathognomonic findings include retinal nerve fiber layer (RNFL) swelling around the disc without leakage on fluorescein angiography, described as pseudoedema, and peripapillary telangiectatic microangiopathy.[1,8] Fibers within the papillomacular bundle temporal to the optic disc are selectively lost in the early stages of the pathologic progress.[3] The eventual loss of retinal ganglion cells results in RNFL thinning in the subsequent months.[9] Histopathologic analyses have characterized degeneration of retinal ganglion cells and their axons leading to clinically apparent optic atrophy.[2,3]

The use of optical coherence tomography (OCT) has led to developments in quantifying the natural progression of peripapillary RNFL changes and ganglion cell/inner plexiform layer (GC-IPL) loss of acute LHON.[1,5] The characteristic peripapillary RNFL changes have been described as early swelling followed by late thinning.[1,10] In particular, the temporal and inferior quadrants experience early swelling while the superior and nasal quadrants tend to have persistent swelling even up to three months after disease onset.[1,11] The GC-IPL has demonstrated a pattern of thinning even pre-symptomatically that mirrors the loss of fibers in the papillomacular bundle, with GC-IPL thickness reaching a minimum six to twelve months after conversion.[2,5,12]

These changes manifest as loss of visual acuity and color vision as well as dense central or cecocentral scotomas visible on visual field testing.[4] However, partial visual recovery has been reported in 4-40% of eyes with idebenone use, mutation, age of onset, and progression rate as dependent factors.[4,13] While various studies have described the unique course of structural and functional changes in LHON, the correlations between structure and function remain inconclusive.[2,3,5]

Through this cross-sectional study, we aim to demonstrate the predictive potential of structural measurements on OCT for visual function in patients with LHON. Since peripapillary RNFL measurements capture superimposed elements of swelling and atrophy leading to confounding ambiguity, our hypothesis is visual function correlates better with the ganglion cell layer due to its pattern of longitudinal thinning.

METHODS

Patient and Data Collection

This study was a retrospective, cross-sectional study of patients presenting with a diagnosis of LHON at UCLA Doheny Eye Institute between August 2021 to August 2022.

Patients testing positive for LHON mutations were included. The thickness measurements of the ganglion cell layer average (GC average), ganglion cell layer minimum (GC minimum), and RNFL layer average were obtained from Cirrus OCT. Pattern of vision loss and mean deviation from visual field testing was obtained from automated perimetry testing via 30-2 Humphrey Visual Field testing. Visual acuity, Ishihara color testing, intraocular pressure, use of idebenone at time of visit, and demographic data were obtained. The number of months between symptom onset and time of visit were also obtained for symptomatic patients.

Patients without confirmatory testing for LHON and those with incomplete data sets were excluded.

Statistical Analysis

Ordinary least squares regressions were performed with visual functions, best corrected visual acuity (BCVA) and visual field mean deviation, as dependent variables. Structural measurements of GC average, GC minimum, and RNFL average were used as independent variables.

Multivariable linear regressions were also performed for each visual function with structural

measurements as independent variables. To rule out multicollinearity amongst independent variables, it was ensured that the Pearson's coefficient between variables was less than 0.7. Additionally, each independent variable was tested for collinearity assumptions, including a tolerance greater than 0.1 and variance inflation factor less than 10.

Output for both simple and multivariable regression included an R square and adjusted R square, accounting for differences in units. The standardized coefficient, Beta, and its corresponding P-value was recorded for each dependent variable in the multivariable regression analysis. A primary analysis as described was conducted for all patients. Simple regressions were completed for asymptomatic and symptomatic patients as subgroups of interest. Multiple regression analysis was conducted for only the symptomatic group to ensure Peter's rule of 10 observations per covariate was met.

Snellen visual acuity was converted to Logarithm of the Minimum Angle of Resolution (LogMAR) for analysis.[14] $P<.05$ was accepted as statistically significant. All statistical analyses were performed using SPSS Version 26.

RESULTS

Study Population Characteristics

A total of 92 eyes from 46 patients were included in the study. A summary of baseline demographic data is outlined in Table 1. Most patients were male (72%). The mean age at time of initial visit was 31.2 years (range: 13-68 years). The most common mitochondrial mutation identified was m.11778G>A (76.1%), followed by m.3640G>A (13.0%), other mutations (6.5%) and m.14484T>C (4.3%). Among symptomatic patients, 72% were on idebenone treatment.

Table 1. Key Demographic and Clinical Data

	N=92 eyes		
Demographics			
Age (y)	31.2		
Sex (% Male, n)	72% (66)		
Mutation (%, n)			
11778	76.1 (70)		
3460	13.0 (12)		
14484	4.3 (4)		
Other	6.5 (6)		
Structural and Functional Vision Measurements	**Total N=92 eyes**	**Symptomatic N=78 eyes**	**Asymptomatic N=14 eyes**
Central Scotoma (%, n)	81.5 (75)	96.15 (75)	0, 0
Visual Field Mean Deviation, mean (SD)	-16.04 (11.64)	-18.68 (10.62)	-1.29 (1.90)
Ganglion Cell Layer Average, mean (SD)	57.5 (14.2)	53.7 (10.73)	78.42 (13.17)
Ganglion Cell Layer Minimum, mean (SD)	48.5 (16.1)	44.41 (11.78)	71.43 (17.8)
RNFL, mean (SD)	69.7 (23.1)	64.7 (20.0)	97.1 (20.0)
Ishihara, mean (SD)	3/14 (+/- 4/14)	2/14 (+/- 2/14)	12/14 (+/- 2/14)
BCVA, LogMAR (SD)	1.08 (.73)	1.27 (.62)	.01 (.06)
IOP, mmHg, mean (SD)	15.0 (3.6)	14.9 (3.7)	15.9 (2.6)
Idebenone (%, n)	60.9 (56)	72 (56)	0 (0)
Time since symptom onset, months (SD)		96.5 (105.4)	

Among all eyes, 85% (N=78 eyes) were symptomatic at the time of visit with an average of 96 months (8 years) after symptom onset. Of the symptomatic patients, 85% (N=66) were seen after 9 months of conversion, the established period for disease stabilization.[15]

Patients had an average best corrected visual acuity of LogMAR 1.08 (~Snellen 20/200), with an average of 3/14 on Ishihara color plate testing. The average mean deviation on visual field testing was -16.04 dB with 81.5% demonstrating a cecocentral scotoma. Thinning was observed in the average RNFL (mean 69.7 microns, normal 80-120 microns), average ganglion cell (mean 57.5 microns, normal 76-88 microns), and minimum ganglion cell layer measurements (mean 48.5 microns, normal 74-86 microns).[16] A summary of structural and visual function measurements for asymptomatic, symptomatic, and all patients are also outlined in Table 1.

A total of 19 patients were excluded for incomplete OCT measurements (2), incomplete functional testing (12), and incomplete genetic testing (5).

Linear Regression Analysis

For simple linear regressions of BCVA, adjusted R square was highest for GC minimum (0.396), followed by GC average (0.248) and RNFL thickness (0.192). When examining visual field mean deviation, adjusted R square was highest for GC minimum (0.320), followed by RNFL thickness (0.223), and GC average (0.216). Standardized beta coefficients and R square values are shown in Table 2.

Table 2. Simple Linear Regression

All		**GC Minimum**	**GC Average**	**RNFL Thickness**
BCVA	R^2	0.403	0.257	0.201
	Adjusted R^2	0.396	0.248	0.192
	Beta	-0.632	-0.507	-0.449
Visual Field (Mean Deviation)	R^2	0.327	0.224	0.231
	Adjusted R^2	0.320	0.216	0.223
	Beta	0.572	0.474	0.481
Asymptomatic		**GC Minimum**	**GC Average**	**RNFL Thickness**
BCVA	R^2	0.186	0.002	0.105
	Adjusted R^2	0.118	-0.081	0.030
	Beta	-0.431	0.043	0.324
Visual Field (Mean Deviation)	R^2	0.090	0.000	0.006
	Adjusted R^2	0.015	-0.083	-0.076
	Beta	0.301	0.006	-0.080
Symptomatic		**GC Minimum**	**GC Average**	**RNFL Thickness**
BCVA	R^2	0.225	0.045	0.047
	Adjusted R^2	0.215	0.032	0.034
	Beta	-0.474	-0.212	-0.217
Visual Field (Mean Deviation)	R^2	0.174	0.053	0.097
	Adjusted R^2	0.163	0.041	0.085
	Beta	0.417	0.231	0.311

The scatterplots for each simple linear regression are shown in Figures 1 and 2 with the trendlines for all patients, asymptomatic, and symptomatic groups displayed. Figure 1 shows BCVA as the dependent variable with each structural measurement as independent variables. Similarly, Figure 2 shows the scatterplots with visual field mean deviation as the dependent variable.

Figure 1. BCVA v. Layer Thickness Scatterplots

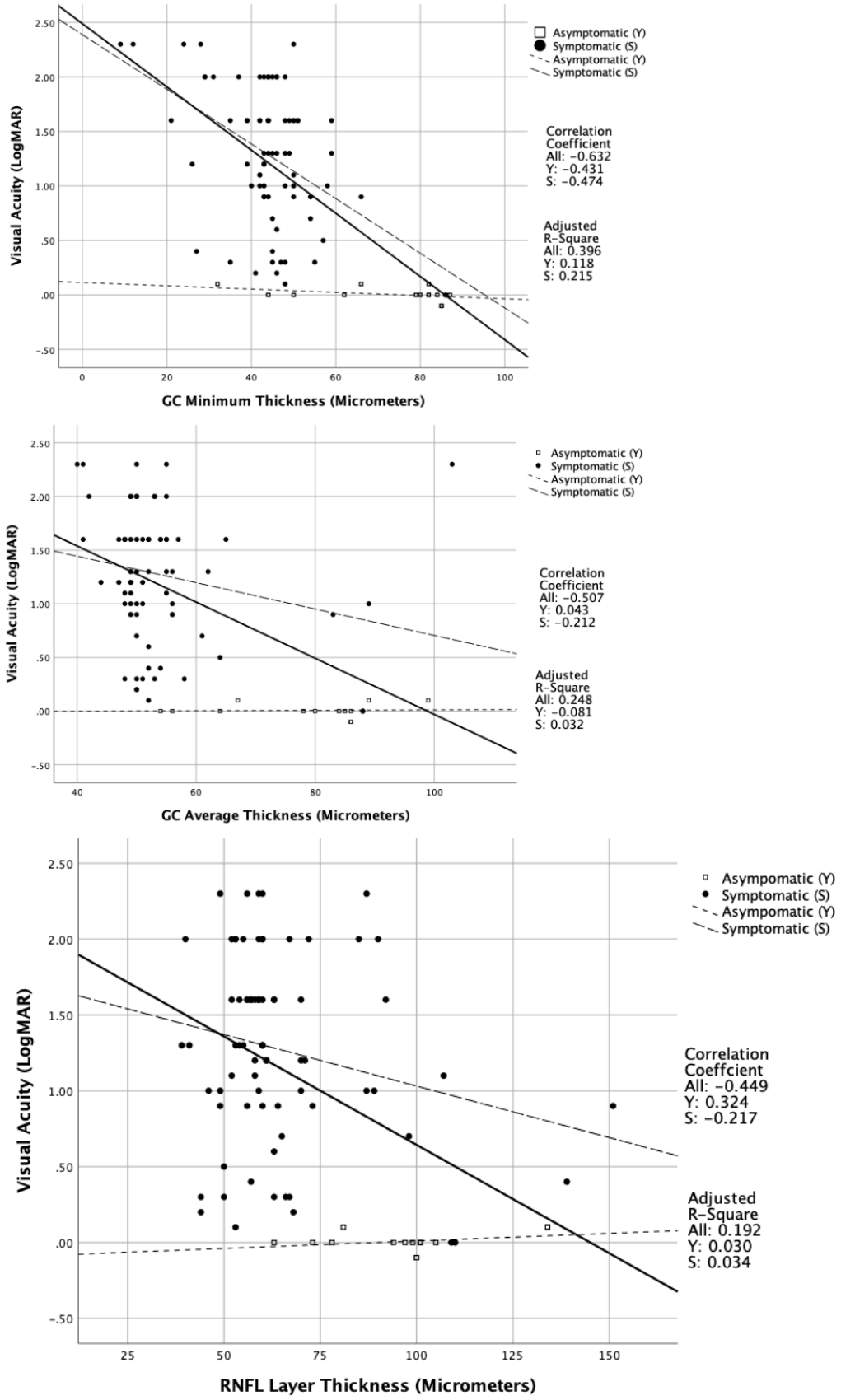

Figure 2. Visual Field Mean Deviation v. Layer Thickness Scatterplots

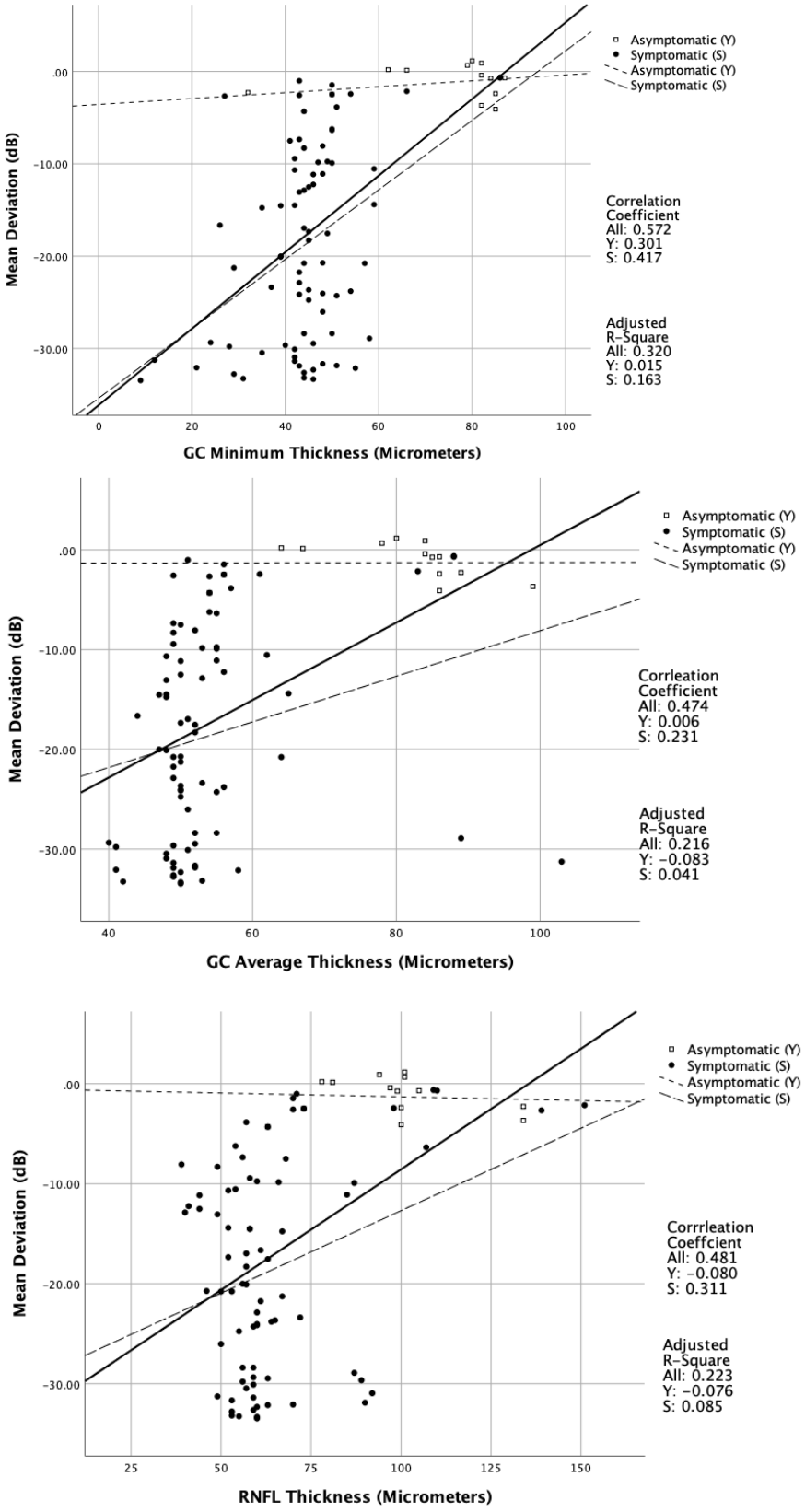

Table 3. shows the multivariate regression of BCVA had an adjusted R^2 of 0.407 with GC minimum as a significant independent variable (Beta -0.527, P<0.001). The adjusted R^2 was 0.207 when evaluating only symptomatic patients. Notably, GC minimum remained a significant variable (Beta -0.479, P<0.001).

Table 3. Multiple Linear Regression

Multiple Regression		**All (N=92)**		**Symptomatic (N=78)**	
		BCVA	**Visual Field (Mean Deviation)**	**BCVA**	**Visual Field (Mean Deviation)**
GC Min	Beta	-0.527	0.440	-0.479	0.387
	P-Value	<0.001	<0.001	<0.001	.002
GC Average	Beta	-0.042	0.004	0.058	-0.041
	P-Value	0.742	0.976	0.638	0.739
RNFL	Beta	-0.157	0.258	-0.094	0.210
	P-Value	0.145	0.023	0.408	0.070
R^2		0.427	0.378	0.238	0.223
Overall Adjusted R^2		0.407	0.356	0.207	0.191

Visual field mean deviation had an adjusted R^2 of 0.356. When evaluating all patients, the standardized coefficient of GC minimum (Beta 0.440, P <0.001), and RNFL (Beta 0.258, P=0.023) were significant. When evaluating only symptomatic patients, the standardized coefficient of GC minimum (Beta 0.387, P=0.002) was significant with RNFL (Beta 0.210, P=0.07) approaching significance. There was no collinearity between independent variables.

DISCUSSION

Our study demonstrated that amongst structural measurements determined on OCT, the minimum ganglion cell layer thickness had the highest correlation with BCVA and visual field function when compared to average thickness of the RNFL and ganglion cell layers. In multivariate regression analysis, the minimum ganglion cell layer continued to account for the highest, and significant bearing on visual acuity and visual field performance. These findings continued to be affirmed when examining only the subgroup of symptomatic eyes.

This confirms our hypothesis that the structural integrity of the ganglion cell layer correlates best with visual functions. Various studies have identified longitudinal changes, which include RNFL swelling, GC-IPL thinning, and macular thinning, in LHON that can even precede visual changes.[1,3,5,9] Our study demonstrated that RNFL does not correlate as well as the minimum ganglion cell measurements in visual acuity, but was significantly correlated to visual field function in our multivariate model. RNFL axons can swell initially followed months later by thinning due to atrophy that produces opposite measures that are superimposed leading to confounding ambiguity.[10] The variability in RNFL thickness makes it less helpful in correlating with visual function compared to the progressive loss of the retinal ganglion cell layer in LHON. This explanation is further supported by weaker associations between visual function and the average ganglion cell layer, a measurement that includes the RNFL layer and subjected to its variability.

A previous study of LHON patients on idebenone found associations between structural measurements and visual function to be stronger at the study end point.[2] It was theorized that retinal ganglion cells are both lost and inactivated in acute phases of LHON. The long-term use of idebenone may mitigate neuronal degeneration or promote the activation of dormant retinal ganglion cells, which permitted for stronger associations between structural measurements and function as the study progressed.[2,17] Notably, these findings and our study capture similar moments in disease course as the majority of our symptomatic patients were also on idebenone (72%) and seen after disease stabilization (85%). Our findings in conjunction with past studies suggests that the minimum ganglion cell layer after treatment maybe an indicator of ganglion cell loss severity that warrants further study.

The study also found that the ganglion cell layer volume at baseline is significantly associated with visual acuity recovery after taking idebenone. The rationale is that greater ganglion cell volume signifies a greater quantity of retinal ganglion cells that have potential for repair and reactivation.[2] Thus if a smaller minimum ganglion cell layer measurement is suggestive of greater ganglion cell loss, we believe it may also be associated with less potential for visual recovery. Future studies can

elucidate whether the minimum ganglion cell layer is associated with less visual gain potential with treatment.

We believe that better understanding the relationships of structural measurements can further improve the prediction of visual function and disease severity. For example, we presume that subtracting RNFL thickness from the minimum ganglion cell thickness could better represent the severity of retinal ganglion loss better than an isolated measurement of GC-IPL. The GC-IPL measurement on OCT includes the inner plexiform layer and the RNFL layer. This proposed difference between GC minimum and RNFL thickness could demonstrate the loss of ganglion cells better ganglion cell layer measurements as the other measurements remain subjected to the variability of the RNFL layer due to its concomitant swelling and atrophy.[5] We remain intrigued as to whether the computation of these structural measurements can demonstrate stronger correlations with visual function than what we found with this investigation.

Our study's cross-sectional design limits its ability to capture the natural history pattern of LHON. Due to the rarity of this disease, our study is also not powered to provide other subgroup analyses based upon known prognostic characteristics such as age or pathologic mutation.

CONCLUSION

Among the OCT structural measurements that were evaluated, the minimum ganglion cell layer thickness emerged as a significant and the best predictor of visual function. Visual field function correlated significantly with both minimum ganglion cell layer and RNFL thickness. These results encourage further study of the minimum ganglion cell layer thickness for its predictive and prognostic potential in LHON.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

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