Male Prevalence of Acquired Color Vision Defects in Asymptomatic Carriers of Leber's Hereditary Optic Neuropathy

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Purpose. Leber's hereditary optic neuropathy (LHON) is a maternally inherited disease resulting in loss of central vision and dyschromatopsia, caused by mitochondrial DNA point mutations. However, only a subset of the mutation carriers becomes affected, with a higher penetrance in males. This study was conducted to investigate chromatic losses in asymptomatic carriers of the LHON mutation.

Methods. Monocular chromatic discrimination was studied with the Cambridge Colour Test (CCT; Cambridge Research Systems, Ltd., Rochester, UK) along the protan, deutan, and tritan cone isolation axes in 46 LHON carriers (15 men) belonging to the same LHON maternal lineage and 74 age-matched control subjects (39 men). Inclusion criteria were absence of ophthalmic complaints and clear ocular media. A detailed neuro-ophthalmic examination was performed in all the LHON carriers.

Results. The differences in threshold between carriers and control subjects were significant for the three cone isolation axes at $P < 0.0001$. Sixty-five percent of the carriers had abnormal protan and/or deutan thresholds; some of those with higher thresholds also had elevated tritan thresholds (13%). The male thresholds were higher and more frequent than those of the women for the protan and deutan axes (ANOVA; $P < 0.05$), but not for tritan thresholds. In the most severe losses, the women had instances of diffuse defect whereas all the men displayed a red–green defect.

Conclusions. Male LHON asymptomatic carriers had color vision losses with the red–green pattern of dyschromatopsia typical of patients affected with LHON, which includes elevation of tritan thresholds as well. This predominantly parvocellular (red–green) impairment is compatible with the histopathology of LHON, which affects mostly the papillomacular bundle. In contrast with male losses, female losses were less frequent and severe. These gender differences are relevant to understanding LHON pathophysiology, suggesting that hormonal factors may be of great importance. (*Invest Ophthalmol Vis Sci.* 2007;48:2362–2370) DOI:10.1167/iovs.06-0331

Leber's hereditary optic neuropathy (LHON) is a maternally inherited disease that causes permanent and bilateral loss of central vision and dyschromatopsia.[1,2] First described by Theodor Leber, it was demonstrated, a century later, to be due to point mutations in the mitochondrial (mt)DNA.[3] Large worldwide case series indicate that more than 90% of LHON pedigrees have one of three mtDNA pathogenic mutations at nucleotide positions 11778G→A/ND4, 3460G→A/ND1, and 14484T→C/ND6, all of them involving genes encoding complex I subunits of the respiratory chain.[1,2]

The elucidation of the genetic basis of LHON allowed important insights into the disease pathophysiology. However, many features remain poorly understood. These enigmas include incomplete disease penetrance, which can be very variable, even in different branches of the same pedigree[4]; male prevalence; and the rather selective degenerative target for the papillomacular bundle (PMB) of the retinal ganglion cell nerve fibers.[1,2] In a typical scenario, approximately 30% to 50% of men and 10% to 15% of women among the maternally related individuals at risk, usually carrying a homoplasmic LHON pathogenic mutation, become affected.[5] In an attempt to resolve these questions, environmental triggers such as diet, smoking, alcohol consumption, and exposure to pollutants[6–8] have been investigated. It has also been hypothesized that modification of nuclear genes may be relevant.[9] Both avenues of research have so far been inconclusive in providing definitive answers.

Recently, attention has been focused on the study of asymptomatic LHON mutation carriers who exhibit subclinical losses in visual function[10–12] and alterations in retinal nerve fiber layer (RNFL) thickness.[13] Therefore, it is relevant to examining the issues of penetrance and clinical expression to identify all sub- or preclinical signs and defects of visual function that may immediately precede and/or predict the onset of the acute, symptomatic phase of the disease. In fact, it is currently not clear whether the asymptomatic carriers of an LHON mutation

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showing defects in visual function will actually become affected.

Dyschromatopsia in LHON has been described in affected patients as a predominantly red–green defect. Color vision studies in patients with LHON performed in the 1960s and 1970s classified the losses as a Verriest type II color defect—that is, a predominantly red–green defect, with concomitant blue–yellow loss in its more severe form. This type of loss is common in diseases of the optic nerve.[14–16]

Asymptomatic maternal relatives of LHON-affected patients (i.e., carriers of the LHON mutation), had normal scores in the FM 100 Hue and in the Ishihara and HRR plate tests, in a study that reported alterations in the early phase of LHON.[17] Other studies reported color vision impairment, among other functional losses, in unaffected carriers.[18–20] However, the number of subjects investigated by these studies was limited, and the molecular definition of the mtDNA mutation was not available at that time. Furthermore, the color vision assessment in carriers of LHON was performed by clinical tests that are semiquantitative, do not use precise psychophysical procedures, and are influenced by confounding factors such as learning and motivation.[15] When compared with recently developed computerized tests that are quantitative and employ a rigorous psychophysical method, they perform with lower sensitivity in the detection of subtle color defects.[21–23]

In LHON, loss of the PMB in the RNFL leads to the predominant symptoms of central scotoma and dyschromatopsia. Hence, the investigation of possible subclinical losses of color vision may be informative regarding the natural history and pathogenic mechanisms of the disease.

We therefore measured color discrimination in a large group of asymptomatic maternal relatives, all belonging to an extended LHON pedigree from Brazil and carrying the homoplasmic 11778G→A/ND4 mutation on a haplogroup J mtDNA background.[8] We used a highly sensitive computerized color vision test, the Cambridge Colour Test (CCT; Cambridge Research Systems, Ltd., Rochester, UK), which allows simultaneous testing of the parvocellular and koniocellular visual pathways, by measurement of thresholds in cone isolation for the protan, deutan, and tritan confusion lines.[21–23] Such probing of the pathways through cone thresholds relies on the assumption that the responses of ganglion cells from the parvo- and koniocellular pathways result from linear combinations of cone inputs, which has been proposed in different studies.[24–26] We also examined possible gender differences in the color vision of these asymptomatic carriers of the LHON mutation.

METHODS

Participants

All the subjects recruited for the present study were inhabitants of the cities of Colatina, Santa Tereza, and Vitória in the state of Espírito Santo, Brazil. They were all molecularly tested and defined as asymptomatic carriers of the homoplasmic 11778G→A/ND4 LHON mutation (henceforth defined as "asymptomatic carriers"), on a haplogroup J mtDNA background, as previously reported.[8,27] They all belonged to a single pedigree that we had recently reported, with 326 members, of which 265 were examined.[8–12] Their identification was accomplished in 2001 to 2002 by an international multidisciplinary team of investigators that has returned to the location yearly and analyzed genetic, biochemical, ophthalmic, neuro-ophthalmic, electrophysiological, and psychophysical aspects of the disease. The data presented herein were collected during the 2003 and 2004 field investigations. The examinations performed in the carriers included computerized perimetry (SITA Standard W-W 24-2 strategy) using an automated perimeter (Humphrey Field Analyser II; Model 750*i*; Carl Zeiss Meditec, Inc., Dublin, CA) and RNFL analysis by laser ophthalmoscopy (GDx; Carl Zeiss Meditec, Inc.). The GDxVCC is a scanning laser polarimeter that indirectly measures the thickness of the RNFL, with a near-infrared laser that makes use of the birefringent properties of the RNFL.

Complete ophthalmic and neuro-ophthalmic examination were performed in all the subjects, to eliminate confounding diseases, such as cataracts, retinopathy, or neuropathy. These examinations included slit lamp examination of anterior chamber with the LOCS II scoring system (Lens Opacity Classification System II)[28] measurement of intraocular pressure, and red-free high intensity ophthalmoscopy. Visual acuity (VA) was measured at 3 m using an Early Treatment Diabetic Retinopathy Study (ETDRS) logMAR (logarithm of the minimum angle of resolution) tumbling-E chart (Lighthouse International, New York, NY).[29] This study adhered to the tenets of the Declaration of Helsinki. Informed consent was obtained from the subjects after explanation of the nature of the study.

We tested color vision in 46 asymptomatic carriers (15 men, 31 women; age range, 18–63 years) and in 74 age-matched control subjects (39 men, 35 women; age range, 19–63 years). Best corrected VA in all control subjects was 20/30 or better in each eye and in the asymptomatic LHON carriers was 20/30 or better in 40 of the 46 participants, 20/40 in 4, and 20/50 in the other 2. Tables 1 and 2 present demographic data of control subjects and of asymptomatic carriers of LHON, respectively. Inclusion criteria were clear ocular media and absence of known neurologic disorders or ophthalmic disease. Fundoscopy revealed subclinical alterations in LHON carriers, which included microangiopathy and nerve fiber swelling.[12] Control subjects were recruited among staff and students from the University of São Paulo. A previous study showed[22] that the test used is not influenced by regional variables involving life habits by showing that color vision thresholds measured in staff and students of the University of São Paulo did not differ from those of healthy subjects from the Amazon region.

Equipment and Procedure

Color discrimination thresholds were measured with the CCT (ver. 2; Cambridge Research Systems Ltd.), run on a microcomputer (Dell Systems, Round Rock, TX), with the VSG 2/5 graphics card and a gamma-corrected color monitor (FD Trinitron model GMD-F500T9; Sony, Tokyo, Japan). This is a computerized test, conceived in the early 1990s, that has been used in many studies.[21–23,30–37]

The visual stimulus consisted of a Landolt C target on a background of different chromaticity,[19,20] presented 3 m from the subject (Fig. 1A). At that distance, the Landolt C gap size corresponded to 1.25° of visual angle, the outer diameter 5.4°, and the inner diameter 2.75°. Both the target and the background were made up of small, discrete patches of variable size (0.5–2 cm in diameter, or 0.05–0.38° of visual angle) and variable luminance (six equal steps between 8 and 18 cd/m^2). The background chromaticity was fixed at a single neutral value (coordinates $u' = 0.197$, $v' = 0.469$ in the 1976 CIE [Commission Internationale de l'Éclairage] u',v' Chromaticity Diagram). The opening of the C was presented in four orientations (up, down, left, or right) and varied randomly from trial to trial. The subject's task was to indicate the position of the Landolt C opening by pressing the corresponding button in a response box. The chromaticity of the target was varied along a vector in color space to determine a discrimination threshold. All subjects were tested in the dark, monocularly, in one randomly selected eye, and performed the test with refractive correction. Correct performance in the test required foveal fixation.

We used the CCT Trivector test (Cambridge Research Systems, Ltd.) to measure independent thresholds along the protan, deutan, and tritan axes. The CRT gamut for the monitor is represented by the colored area in the section of the CIE 1976 u',v' diagram in Figure 1A, in which the CRT phosphor limits were: red phosphor (R) $u' = 0.416$; $v' = 0.522$; green phosphor (G) $u' = 0.117$; $v' = 0.559$; blue phosphor (B) $u' = 0.159$; $v' = 0.177$ in u',v' chromaticity coordinates.

Thresholds were measured with a psychophysical staircase procedure that started with a target chromaticity at the extreme of CRT

Table 1. Demographic Data and Results: Control Subjects

Subject	Age	Gender	Color Discrimination Threshold			Subject	Age	Gender	Color Discrimination Threshold		
			Protan	Deutan	Tritan				Protan	Deutan	Tritan
92	19	M	33	40	57	99	19	F	75	61	90
94	19	M	44	56	86	135	19	F	44	30	58
102	19	M	29	30	44	144	19	F	62	81	82
128	19	M	47	49	71	100	20	F	35	29	29
129	19	M	33	40	88	101	20	F	62	53	72
160	19	M	46	29	65	133	20	F	52	46	49
108	20	M	41	52	55	103	21	F	46	47	47
90	21	M	43	38	70	104	21	F	33	23	65
109	21	M	46	41	56	125	21	F	49	54	85
106	22	M	46	46	75	126	21	F	35	41	67
113	22	M	32	29	45	105	22	F	53	50	79
137	22	M	47	41	78	107	22	F	36	36	39
110	23	M	29	30	44	122	22	F	49	43	47
114	23	M	62	53	86	159	22	F	38	30	36
116	23	M	52	43	112	111	23	F	36	38	82
119	23	M	47	40	84	156	23	F	57	47	85
117	24	M	62	53	86	118	24	F	46	29	69
96	25	M	57	49	52	121	26	F	26	35	70
120	25	M	29	29	52	134	29	F	51	54	53
130	28	M	33	45	77	95	30	F	53	78	87
139	29	M	47	54	47	98	31	F	26	29	45
97	30	M	44	56	86	132	31	F	26	29	61
140	31	M	45	49	84	178	32	F	53	54	79
138	32	M	41	54	98	149	35	F	67	52	142
181	33	M	79	53	119	184	37	F	57	88	145
183	34	M	36	54	70	185	37	F	63	43	88
182	36	M	39	56	79	191	41	F	43	50	91
186	36	M	46	44	49	151	42	F	49	77	93
115	37	M	33	40	88	147	44	F	113	140	204
148	37	M	52	29	58	146	45	F	52	29	58
179	37	M	59	29	63	165	55	F	120	87	196
188	40	M	61	73	88	155	60	F	74	95	102
190	45	M	66	59	62	161	60	F	71	70	154
167	48	M	82	71	163	174	62	F	75	61	90
168	52	M	36	41	36	166	63	F	51	41	143
164	55	M	44	51	167						
162	58	M	56	88	136	MEAN	32		50	50	81
163	59	M	52	52	93	SD	13		17	17	36
171	61	M	51	86	56						

gamut for each of the three confusion axes (P, D, and T; Fig. 1A) and ended at the threshold. The distance between the target and background chromaticities along the corresponding vector was decreased for correct responses and increased for incorrect responses. Staircase step size was changed by using an adaptive procedure, a linear staircase optimized for the CCT (proprietary algorithm from Cambridge Research Systems, Ltd.).

The CCT software measured the three thresholds simultaneously by interleaving the three staircases randomly. Response reliability was monitored by interspersed presentation of catch trials with maximum chromatic saturation. A threshold computed at each staircase after six reversals had occurred expressed the average chromaticity defined by the reversals. The test lasted 3 to 5 minutes.

Intraobserver and Test–Retest Reliability of the CCT

The CCT incorporates a reliability testing procedure with catch trials that present a saturated color at the maximum of the CRT gamut (95% CIE 1976 coordinates: $u' = 0.119$; $v' = 0.391$; vector length = 1100 $u'v'$ units). This procedure tests for the ability of the subject to respond correctly to the target, which depends on the understanding of instructions and on the attention directed to the task during the testing session. We checked this in control subjects and carriers of LHON. The reliability measured in this way was 100%.

Test–retest reliability for the CCT was measured in recent work from our group, which tested for learning effects, dominant versus nondominant eye and binocular versus monocular measurements. No significant differences were found for any of these conditions (i.e., test–retest reliability for the CCT is high), and we found no evidence of learning or fatigue effects.[37]

Statistical Analysis

Subjects were grouped according to sex within asymptomatic carriers and control groups. One randomly chosen eye was considered for the analysis. Statistical analysis was performed with commercial software (StatSoft ver. 6.0, Statistica Inc., Tulsa, OK). Statistical assessments of differences among groups were performed by one-way ANOVA; by main effect ANOVA, considering the two factors gender and fundus; by Student's *t*-test for independent samples, with a weight correction for different size samples in data normally distributed; and with the Mann-Whitney test when the distribution was not normal. Adhesion to the normal distribution was checked with the Kolmogorov-Smirnov test. The Spearman correlation coefficient was used to quantify the relationship between color vision results and other variables such as age and visual acuity. Tolerance limits were calculated to cover 95% of the population with 95% probability in the total sample, and separately, for stratified male and female data.

TABLE 2. Demographic Data and Results: 11778 LHON Carriers

						CCT threshold				
Patient	**Age**	**Gender**	**Eye**	**VA Snellen**	**Fundus**	**Protan**	**Deutan**	**Tritan**	**Visual Field**	**GDx TSNIT**
1	22	M	OS	20/20	N	54	53	61	−1.5	57.4
2	24	M	OD	20/20	N	197	25	99	−1.25	66.6
3	37	M	OS	20/25	N	134	121	99	−1.25	66.1
4	38	M	OD	20/20	N	89	72	78	−0.5	64.6
5	40	M	OD	20/25	N	134	155	164	−2.25	58.4
6	40	M	OD	20/25	N	80	94	97	−1.5	63.4
7	51	M	OS	20/20	N	188	304	152	−2.75	55.3
8	53	M	OD	20/30	N	466	938	110	−3	68.2
9	39	M	OS	20/20	TP	122	110	128	−1.25	59.6
10	40	M	OS	20/25	TP	68	90	138	−3	56.2
11	45	M	OS	20/20	TP	432	75	206	−1.25	63.6
12	22	M	OD	20/25	TP+SM	99	103	77	1	54.5
13	25	M	OS	20/20	SM	236	251	79	−2	59.3
14	49	M	OD	20/25	SM	69	53	81	−1.75	58.8
15	42	M	OS	20/20	N	88	89	62	−1.75	55.2
16	18	F	OD	20/30	SM	95	218	102	0	58.8
17	19	F	OS	20/20	N	36	70	65	−0.5	58.4
18	20	F	OD	20/25	SM	86	59	106	−1.5	54.4
19	22	F	OS	20/30	N	36	81	77	−2.5	61.8
20	22	F	OD	20/20	N	93	80	111	−3.5	—
21	22	F	OD	20/25	N	49	43	72	−1.25	56.8
22	23	F	OS	20/20	N	97	88	65	−1.75	58.3
23	23	F	OD	20/30	N	71	39	72	−2.25	58.7
24	30	F	OS	20/25	N	356	339	365	−2	51.8
25	30	F	OD	20/20	N	67	70	91	0	55.0
26	31	F	OD	20/25	N	79	84	106	−1.75	55.4
27	34	F	OD	20/20	N	64	53	85	−0.25	55.9
28	35	F	OS	20/20	N	57	44	80	−1.75	62.6
29	35	F	OS	20/40	N	63	32	30	−2.75	50.4
30	35	F	OD	20/25	SM	49	53	55	−4	61.6
31	36	F	OD	20/50	N	91	58	84	−1.25	58.9
32	37	F	OS	20/25	N	140	160	104	−1.5	56.5
33	37	F	OD	20/30	N	127	119	100	−3	60.3
34	42	F	OS	20/25	N	109	53	154	−2	59.7
35	42	F	OD	20/25	TP	166	150	131	−0.5	60.5
36	44	F	OS	20/40	N	212	138	259	−1.75	55.0
37	45	F	OD	20/25	N	55	96	94	−3.25	55.4
38	46	F	OD	20/25	SM	76	45	104	−2	61.0
39	50	F	OD	20/30	N	61	87	129	−2.5	58.7
40	53	F	OS	20/25	N	86	80	123	−3.5	62.5
41	54	F	OS	20/50	N	128	124	156	−1.5	56.3
42	54	F	OD	20/30	N	52	91	142	−0.5	61.9
43	55	F	OD	20/40	SM	50	37	98	−1.75	59.1
44	56	F	OS	20/40	TP	210	140	217	−0.25	58.8
45	58	F	OD	20/30	N	69	94	112	−0.25	59.6
46	63	F	OS	20/30	TP	211	172	215	0.25	55.3
Mean	38					122	125	116	−1.63	58.9
SD	12					95	141	59	1.09	9.4

N, normal; TP, temporal pallor; SM, swelling microangiopathy; TP+SM, temporal pallor+swelling microangiopathy. Visual field values, mean deviations in dB. Tolerance limits: Total sample: protan 89; deutan 88; tritan 163; men: protan 75; deutan 80; tritan 146; and women: protan 99; deutan 95; tritan 181.

The 95% confidence intervals (95% CIs) of the proportions were calculated according to the formula $P \pm 1.96 \cdot \sqrt{(pq/n)}$ where p represents the proportion, $q = 1 - p$, and n is the sample size on which the proportion is based. CIs are provided for all proportions.

RESULTS

Color discrimination losses were detected in 30/46 or 65% (95% CI: 51%–79%) of the total sample of LHON asymptomatic carriers (both sexes). Protan losses occurred in 24/46 or 52% (95% CI: 38%–66%) and deutan in 25/46 or 54% (95% CI: 40%–68%). The losses in the protan and deutan axes were greater than in the tritan axis, in which they were found in 6/46 or 13% (95% CI: 3%–22%) of the carriers (Figs. 1B, 1C). The differences in threshold between carriers and controls were significant for all three axes at $P < .0001$ (one-way ANOVA) (Fig. 1B). Thresholds and significances are presented in Table 3.

Stratifying the data for gender showed that male color vision was more affected than female. A greater percentage of male asymptomatic LHON carriers, 13/15 or 87% (95% CI: 70%–100%), displayed color discrimination losses as compared to females 14/31 or 45% (95% CI: 27%–63%). A protan defect was found in 11/15 of the males or 73% (95% CI: 50%–95%) and 9/31 females or 29% (95% CI: 25%–59%). A deutan defect was found in 11/15 males or 73% (95% CI: 51%–95%) and 13/31 females or 42% (95% CI: 27%–63%). Some

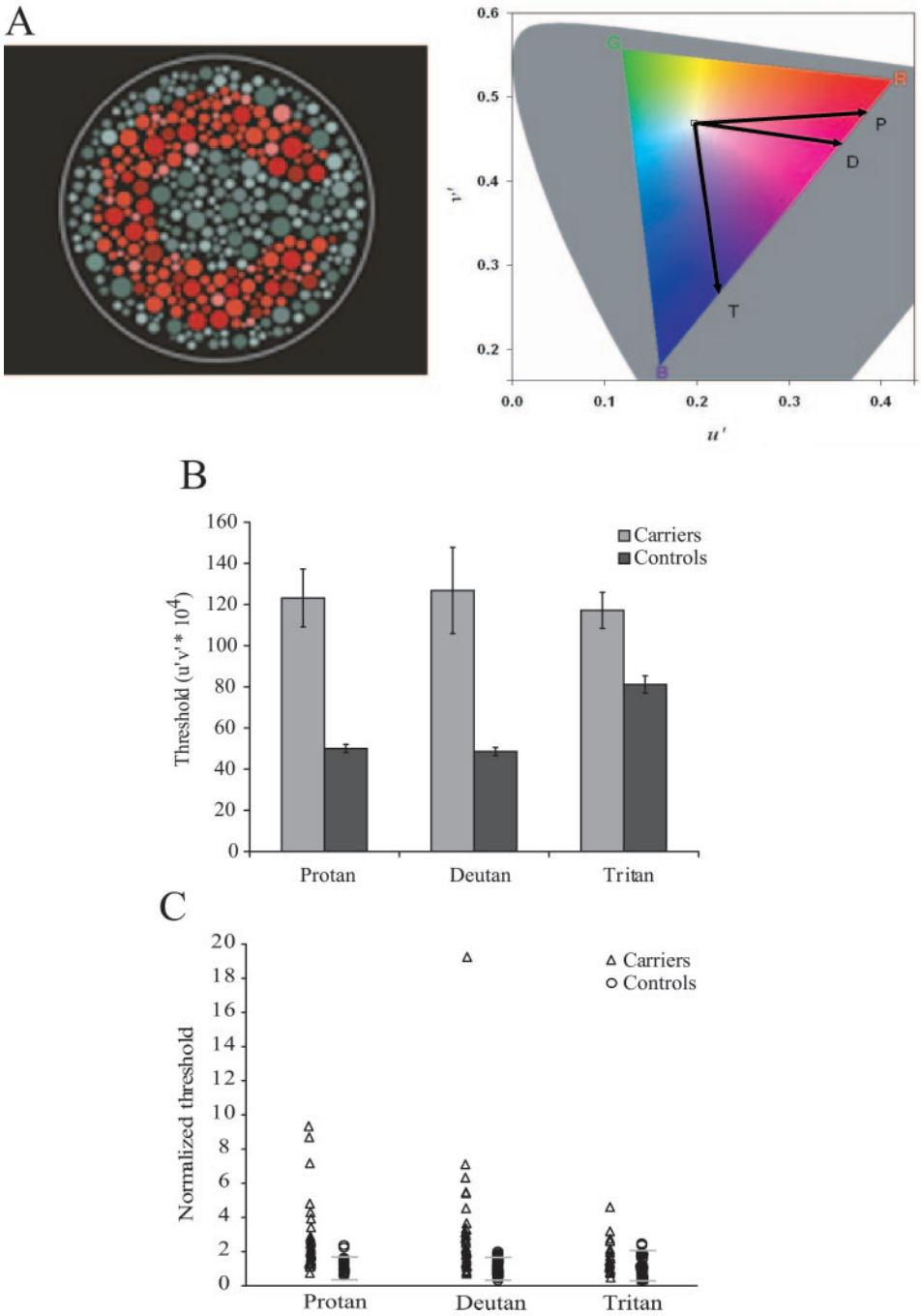

FIGURE 1. (**A**) *Left*: stimulus from the CCT, showing spatial and luminance noise in the background and in the Landolt C chromatic target. The subject's task was to indicate the position of the gap, which is changed randomly among right, left, up, and down from one trial to the next. *Right*: section of the CIE 1976 chromaticity diagram showing the coordinates for the stimuli used in the CCT Trivector procedure. The CRT gamut for the monitor we used is represented by the colored area, which is delimited by the phosphor chromaticities: red phosphor (R) $u' = 0.416$; $v' = 0.522$; green phosphor (G) $u' = 0.117$; $v' = 0.559$; blue phosphor (B) $u' = 0.159$; $v' = 0.177$ in $u'v'$ chromaticity coordinates. The chromaticity of the target (Landolt C) varied along the protan (P), deutan (D), and tritan (T) confusion axes. The background chromaticity was fixed and it was located at the point where the axes converge. Thresholds were determined along each of the confusion axes. (**B**) Average and SE of the thresholds of asymptomatic 11778 LHON carriers ($n = 46$) and control subjects ($n = 74$) in the Trivector test of the CCT for the protan, deutan, and tritan axes. Statistical significance was observed between control subjects and carriers for all three confusion axes. However, threshold elevation was larger for the protan and deutan axes. (**C**) Protan, deutan, and tritan thresholds from the LHON carriers and control subjects, presented in (**B**) were normalized to the respective average value in the control group. (△) Carrier thresholds; (○) control thresholds; *horizontal bars*: tolerance limits.

of the carriers with protan and/or deutan defects also had tritan defects, in 2/15 or 13% (95% CI: 0%–30%) males and 4/31 or 13% (95% CI: 1%–25%) of the females. There were no carriers that had exclusively tritan defects.

Male losses were therefore characterized by a pronounced red–green defect, with higher elevation of both protan and deutan than of tritan thresholds in the CCT Trivector test (Table 2, Fig. 2). The female pattern of color impairment was also predominantly red-green, but less frequent and less severe. A few carriers (4/31 women and 2/15 men) had a diffuse pattern of losses, with elevation of thresholds in all three axes. Accordingly, the statistical comparisons presented in Table 3 show significant differences between male and female results for protan and deutan thresholds, but not for tritan results (main effect ANOVA). Differences between female carriers and control subjects and between male carriers and control subjects were also significant. The protan and deutan thresholds differences were significant at $P < 0.0005$; whereas the tritan thresholds differed at $P < 0.05$ for the women and $P < 0.005$ for the men. No gender difference was found between the men and women in the control group for any of the three axes (main effect ANOVA).

We also looked at the more extreme losses to compare the ratio of male and female asymptomatic LHON carriers who had normalized thresholds >3. Six (40%) of 15 of the men had protan and/or deutan thresholds >3, but none of these or any other male carrier had a tritan normalized threshold >3. All had a selective red–green pattern. Among the female carriers, there were 7 (23%) of 31 with protan or deutan thresholds or both >3. Two of these also had a tritan threshold >3. These

TABLE 3. Average Color Discrimination Threshold* along the Protan, Deutan, and Tritan Confusion Axes

	Protan	Deutan	Tritan
Carriers	121.67	125.22	115.98
Controls	50.07	48.59	81.18
P	<0.0001	<0.0001	<0.0001
Female carriers	101.00	96.68	119.48
Female controls	53.66	49.26	85.20
P	=0.0002	<0.0001	=0.0139
Male carriers	163.73	184.20	108.73
Male controls	46.85	48.00	77.56
P	<0.0001	=0.0003	=0.0034
Female carriers	101.00	96.68	119.48
Male carriers	163.73	184.20	108.73
P	0.0352	0.0469	NS
Female controls	53.66	49.26	85.20
Male controls	46.85	48.00	77.56
P	NS	NS	NS

* $u'v' \times 10^4$ units.

two women showed a diffuse pattern. The remaining five women had elevated protan or deutan thresholds with a tritan threshold <3. Therefore, 5 (16%) of 31 of the women had a selective and larger red–green color loss similar to the men.

Moderate or weak positive correlations (Spearman rank test) between age and color discrimination threshold were found in the control subjects, in both the men ($R = 0.34$, 0.20, and 0.60; $P < 0.05$; respectively, for protan, deutan, and tritan thresholds) and the women ($R = 0.36$, 0.44, and 0.25; $P < 0.05$, respectively for protan, deutan, and tritan thresholds)—that is, color discrimination worsened with age, in accordance with the literature.[38–40] In female LHON carriers, a positive moderate correlation between age and tritan thresholds was found ($R = 0.54$; $P < 0.001$), but there was no significance for the correlations of protan and deutan thresholds with age. For male carriers there was no correlation between age and color discrimination thresholds.

The presence of fundus alterations in asymptomatic carriers, specified in Table 2, was associated with higher tritan thresholds in a comparison of thresholds from subjects with and without fundus alterations (main effect ANOVA $F_{41,2990} =$

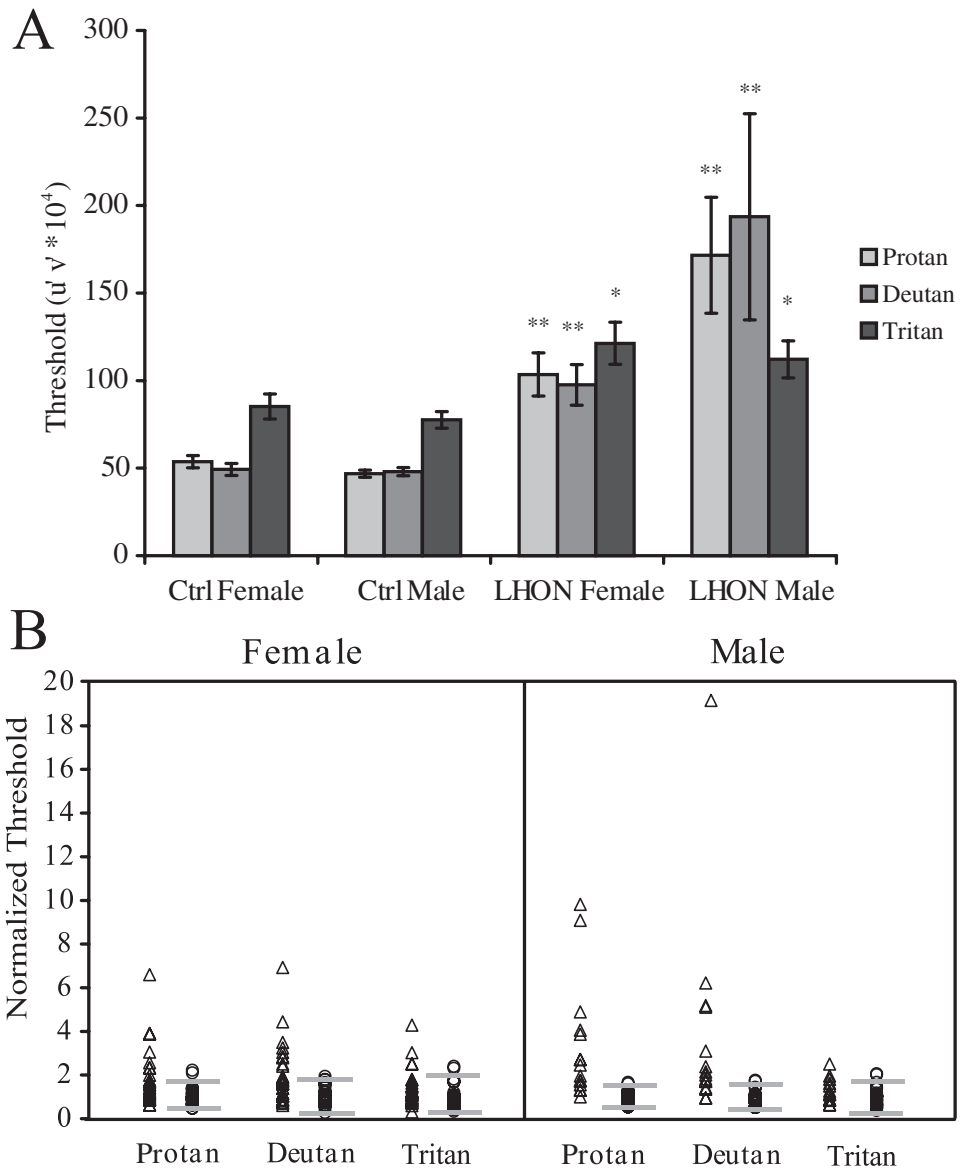

FIGURE 2. (**A**) Mean CCT Trivector test results and standard errors for male ($n = 15$) and female ($n = 31$) asymptomatic 11778 LHON carriers and for the male ($n = 39$) and female ($n = 35$) control subjects. LHON males and females differed significantly from control subjects for all three axes ($P < 0.005$). The LHON-affected men differed from the LHON-affected women for the protan ($P = 0.0352$) and deutan ($P = 0.0468$) axes, but not for the tritan axis ($P = 0.5708$). *Significant difference from the corresponding control (men or women); **significant difference from control and between the men and women. (**B**) Same data as in (**A**), normalized to the corresponding control average. (△) Carrier thresholds; (○) control thresholds; *horizontal bars*: tolerance limits.

3.2549, $P = 0.0311$). In a stratification of the carriers with fundus alterations by sex, the impairment in color discrimination was revealed for the deutan results ($F_{41;19,521} = 4.4602$, $P = 0.0408$). The visual acuity of asymptomatic carriers did not correlate with color discrimination thresholds for any of the Trivector axes. The GDxVCC in the carrier eyes tested with the CCT showed that all had normal GDx scans in the PMB (Table 2). The Spearman correlations between the CCT thresholds and the GDx TSNIT were nonsignificant. Achromatic sensitivity (Table 2) was returned as the average of the four central locations (6°) on visual field measurements, as this corresponds to the area involved in the color discrimination task of the CCT (5.4°). Spearman correlations between visual field sensitivities (average total deviation in the central 6°) and color thresholds were not significant for any of the three color confusion axes, meaning that color discrimination and achromatic sensitivity were not related. When stratified in male and female subgroups, the correlations were also not significant.

Discussion

In the present study, we show marked gender-dependent differences for subclinical losses of visual function in asymptomatic LHON mutation carriers. Incomplete penetrance and sex bias are well known features in LHON[1,2]; however, there is no previous report on gender differences in any visual function of asymptomatic LHON mutation carriers.

The present data show that color vision losses in the asymptomatic LHON carriers reflect the gender bias observed in affected individuals, since a greater percentage of the men had losses. In addition, the male alterations were larger than those found in the women and the pattern closely resembled that of affected patients with LHON tested with the CCT Trivector (Gualtieri M et al. *IOVS* 2004;45:ARVO E-Abstract 4331).

A selective red–green defect had been reported in LHON-affected patients previously, with less sensitive tests used in clinical practice, such as the Farnsworth-Munsell 100 Hue test or the Ishihara plates.[14,17–20] In a group of patients with a variety of optic neuropathies, among which there were two LHON-affected patients, selective red–green losses were confirmed with sensitive psychophysical techniques by Grigsby et al.,[16] who also found blue–yellow losses. However, they did not examine carriers of LHON.

In female carriers of LHON, the losses found in the present study were less severe than in the men, and there was a lower frequency of losses. In the women with severe losses, with thresholds above three times control values, there were also cases of a diffuse effect, with elevated thresholds in all three color confusion axes, while in the correspondingly altered males the defect was restricted to the red–green loss.

It should be remarked that the control group did not show any gender bias in color discrimination and that our experience with several other diseases did not show a gender bias either. For example, we tested 20 male and 20 female nonretinopathic patients with type 2 diabetes, whose color thresholds were significantly elevated, and no gender differences were detected (Gualtieri M et al. *IOVS* 2005;46:ARVO E-Abstract 4750).

We have reported chromatic losses in asymptomatic carriers of the LHON mutation in another chromatic task, equiluminance adjustment through heterochromatic flicker photometry (HFP), and showed that the losses were selective for the red–green system.[10] Asymptomatic carriers required higher luminances in the green compared to control subjects for their equiluminance adjustments. For blue–yellow equiluminance adjustments, there was no difference between asymptomatic carriers and control subjects.

The mechanism for the dyschromatopsia present in the LHON-affected patients is probably related to the fact that LHON first and preferentially affects the thinnest retinal ganglion cells, which constitute the PMB of the RNFL.[2,41] The smallest diameter fibers in the retina belong to the parvocellular neurons (midget ganglion cells). The parvocellular pathway is therefore likely to be the most affected substrate in LHON. The red–green losses found psychophysically in the present and previous studies[14,16,20] are compatible with the histopathological findings,[2,41–43] since red–green color vision is mediated by the parvocellular pathway.[44] Consistent with this view, our functional data show that in the male carriers, who have higher propensity to become affected, the red–green system is much more impaired than the tritan system. The tritan, or S-cone system, is mediated by larger neurons (small bistratified ganglion cells) that form the koniocellular pathway.[44] In the female asymptomatic carriers, the color vision defect was found in a smaller proportion of the carriers and was less severe. The pathophysiological mechanism was also selective for the red–green system in the women, although in a milder way, and the lower frequency of female losses reflects the fact that LHON affects mostly male carriers. It remains unclear as to whether the alterations in color vision of asymptomatic LHON carriers reflect an earlier stage of the disease, or more likely, represent a sort of compensatory adjustment to the subclinical disease.

The fact that asymptomatic carriers present the same type of color vision losses, but in a less severe fashion, as the affected individuals, indicates that the same pathophysiological mechanism is at work in both categories of mutated subjects. It is not surprising that some impairment affects the smallest caliber retinal ganglion cell axons in asymptomatic carriers before the threshold for massive death of these neurons is reached, converting to the acute disease. This early precataclysmic dysfunction has also been demonstrated by a recent optical coherence tomography (OCT) study of asymptomatic carriers of LHON mutations who showed RNFL thickening, possibly due to axonal swelling, in the temporal quadrant where the small papillomacular fibers lie (Savini G et al. *IOVS* 2005;46:ARVO E-Abstract 1202). In agreement with our color vision findings, this study showed that the alterations were more pronounced in the men, who displayed a significant thickening of inferior and temporal quadrants, when compared with the females who had a significant increase in RNFL thickness only in the temporal quadrant.

In contrast, all carriers tested with the CCT in the present study had normal GDxVCC results. The GDxVCC is excellent in early glaucoma detection because the axonal loss in glaucoma is often in the thick superior and inferior arcuate bundles, zones easily measured with this instrument. However, it often fails to detect a difference between the normally thin PMB and the pathologic further thinning that characterizes most optic neuropathies. Because the CCT measures color discrimination thresholds in the central 5.4° of the central visual field and the GDx has difficulty in the corresponding PMB, structure–function discordance between the CCT and GDx in LHON is not surprising and is even predictable. Psychophysical testing of color vision was more sensitive for detecting subclinical losses in carriers of LHON than was the RNFL analysis. By the same token, the average psychophysical light detection thresholds measured in perimetry at the center of the visual field, in the area corresponding to that used in the CCT color discriminations, were within normal values for both the male and female LHON carriers and did not show correlation with the color discrimination findings.

Color vision losses in LHON agree with Kollner's rule that diseases of the optic nerve affect the red–green system, whereas blue (tritan) defects are characteristic of retinal disor-

ders.[45] Agreement with the rule is best explained by the fact that the parvocellular neurons, which mediate red–green color vision, are the most affected in LHON. This pathology therefore provides additional confirmation of the separation between red–green and blue–yellow pathways already demonstrated in human and animal studies.[44,46]

The gender bias in clinically expressed LHON remains unexplained. It is reflected by the male prevalence for color vision losses in asymptomatic LHON mutation carriers as demonstrated herein, as well as for the previously reported RNFL abnormalities observed by OCT.[13]

Historically, the first hypothesis put forward implied a modifying gene on the X chromosome.[1,2] This putative X-linked modifying gene would justify the male prevalence, and its existence was suggested by segregation analysis of LHON families,[47] including the Brazilian family in this study (Carelli V et al. *IOVS* 2003;44:ARVO E-Abstract 937). Over the past years, most attempts to identify a locus and locate the gene on the X chromosome have been inconclusive.[48] However, linkage analyses in our Brazilian family and in a recent large study with European LHON families are again supporting the existence of such X-linked loci (Shankar SP et al. *IOVS* 2005;46:ARVO E-Abstract 663).[49]

The second hypothesis is that the hormonal and metabolic differences between males and females could themselves justify the different gender penetrance.[2] In particular, considering the consistent indications that production of reactive oxygen species (ROS) is increased in LHON,[50,51] the role of estrogens in protecting from oxidative stress may make a difference.[2,52] Overall, our present results and the male prevalence of RNFL abnormalities observed by OCT in asymptomatic mutation carriers may have an alternative explanation in a "hormonal–metabolic" hypothesis, in addition to or rather than an X-linked modifying gene.

Our study, along with others on sub- or preclinical changes in the visual system of asymptomatic LHON mutation carriers,[10–13] indicates that the disease process is active in both asymptomatic and LHON-affected individuals, adding evidence that there is a continuous spectrum of alterations. It is therefore very important to explore prospectively the visual function of the largest number of asymptomatic LHON carriers with the most sensitive methods. The major relevance of the present and other findings of subclinical changes in LHON is the theoretical possibility of a preventive action that could alter the progress of the disease. In future trials investigating potential alternatives for treatment, the evaluation of color vision may provide a useful indicator.

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