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Pan-American mDNA haplogroups in Chilean patients with Leber's hereditary optic neuropathy

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Purpose: The clinical impact of mDNA mutations on the development of Leber hereditary optic neuropathy (LHON) may be modulated by mitochondrial haplogroups, which vary across populations. The aim of this research was to determine the clinical spectrum and molecular characteristics, including the haplogroup, of 15 South American families with LHON.

Methods: This study was a prospective, observational study conducted between March 2006 and August 2012. All patients were referred to the Clinical Hospital of the University of Chile, where the clinical study was conducted. Molecular studies were conducted at the Biomedical Sciences Institute (ICBM) of the University of Chile. Fifteen index cases were identified with molecular analysis after initial neuroophthalmic examination at different centers throughout Chile. Clinical features of patients with LHON and maternal relatives of the 15 families (75 individuals: 26 affected and 49 healthy carriers) were evaluated. The primary mDNA mutations (m.3460G>A, m.11778G>A, or m.14484T>C) were determined with restriction fragment length polymorphism analysis in all individuals. Mitochondrial haplogroups were determined with direct sequencing of two hypervariable regions (HV1 and HV2) and compared with reference sequences.

Results: The m.11778G>A mutation was found in 59 subjects (78.7%), the m.14484T>C mutation was found in 12 subjects (16.0%), and the m.3460G>A mutation was found in four (5.3%) subjects. The average age of onset of symptoms in affected subjects was 22.2 years old (range 3 to 53 years); 21 (80.7%) were male, and five (19.3%) were female. Twelve families (80%) had Amerindian haplogroups: One family had the A2 haplogroup, four families had the B2i2 haplogroup, six families had the C1b haplogroup, and one family had the D1g haplogroup.

Conclusions: In this limited sample size, the Amerindian haplogroup A2 was associated with delayed onset of disease in this population. Patients with haplogroup C retained better vision than the patients with other haplogroups in this population. Disease in subjects with haplogroup D appeared to be underrepresented compared to the population at large.

Leber hereditary optic neuropathy (LHON, OMIM, 535000) is a mitochondrial genetic disorder characterized by bilateral, subacute, painless, and irreversible vision loss, most commonly in previously healthy young men. The severe decrease in vision is characterized by a large central or cecocentral scotoma in the visual field. The worldwide prevalence of LHON is estimated to be between 1:30,000 and 1:50,000. This prevalence varies in different populations and in most populations is unknown [1-5]. The reported prevalence in Finland is 1:50,000 [3], while in northeast England, the prevalence is 1:30,000 [1], and in a Dutch population 1:39,000 [2]. It is felt to be rare in Chinese populations [5]. The prevalence of LHON in Chile is not known.

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The exact pathogenesis of LHON has not yet been fully resolved. Three primary mDNA point mutations, m.3460G>A /MT-ND1, m.11778G>A /MT-ND4, and m.14484T>C / MT-ND6, are found in more than 90% of patients with LHON [6-10]. More than 30 other rare mitochondrial mutations have also been associated with LHON [11,12].

Previous studies have suggested that clinical expression of LHON could be modulated by multiple factors, including nuclear genes and environmental influences [13-15]. Mitochondrial DNA is highly polymorphic, and changes in various positions define haplogroups by the presence of specific combinations of mutations in their sequence. Haplogroups vary by ethnic group, and newer studies have suggested that in addition to nuclear genetics and environmental influences [15,16], the mDNA background, including the haplogroup, affects the clinical expression, penetrance, and prognosis of LHON [2,14,17,18]. In particular, haplogroup J in the European population appears to be linked to higher penetrance of

the m.l4484T>C mutation compared to carriers with different haplogroups.

Before the Spanish arrived in 1520 in what is today Chile, the territory was inhabited by various indigenous groups, including Aymaras, Atacameños, Picunche, Mapuche, Pehuenche, Huilliche, Chonos, Kawéskar, and Yámana [19,20]. During the early period of the Spanish conquest, most immigrants were male. Other significant additions to the population included slaves of primarily African descent, who were brought to Chile until slavery was abolished in 1823 [21-23].

As a consequence of the predominantly male gender of the Spanish emigrants to Chile, asymmetric miscegenation occurred primarily between Spanish men with indigenous women, and to a lesser extent, female slaves of African descent [24]. Thus, there is a peculiar distribution of haplogroups in Santiago's population as a result of this historical occurrence in which female ancestors were mainly Amerindian and male ancestors mainly European [24].

There are four predominant haplogroup founders in South America, which are designated A, B, C, and D [25-28]. In Chile, 37 haplogroups have been described that can be grouped into a large number of sub-haplogroups [26-31]. Studies show that the frequency of haplogroup B decreases from north to south within Chile. In turn, haplogroups C and D increase in frequency from north to south [26-32]. Because the variety of human mDNA haplogroups is directly related to ethnicity, studies showing the influence of haplogroup background on LHON are often limited to the specific populations in which the study was conducted [2,4,13,33-37].

In Latin America, several studies have been conducted in families with LHON, but the families primarily had European haplogroups [36-39]. No studies to date have correlated South American mitochondrial haplogroups with clinical manifestations in patients with LHON. The main aim of this study was to report the molecular and ophthalmic clinical characteristics of 15 Chilean families with LHON and to relate the characteristics to the mitochondrial haplogroups.

METHODS

Participants: 75 individuals, 26 affected (21 male and 5 female) and 49 healthy (12 male and 37 female) were recruited from six different hospitals throughout Chile. All examinations were performed according to the tenets of the Declaration of Helsinki, and the present study was approved by the ethics committee of the Clinical Hospital of the University of Chile and was adhered to the ARVO Statement. This study was conducted between March 2006 and August 2012. All participants gave written informed consent to participate in the study. Index cases were identified during neuroophthalmic examination at different centers throughout Chile. All patients were referred to the Clinical Hospital of the University of Chile, where a clinical record was obtained, pedigrees were constructed, and genetic testing was performed.

All patients and available relatives were interviewed. Patients were questioned regarding the characteristics of visual loss, as well as previous habits before and after the onset of the disease. Smoking at least one cigarette daily within 6 months of symptom onset as well as during or after the appearance of the disease was considered positive for tobacco exposure. Any history of exposure to alcohol, illegal drugs, or industrial toxins was also recorded.

In each case, the clinical suspicion for LHON was first identified by a neuroophthalmologist who did not know the individual's genetic status. After genetic testing, all individuals with bilateral optic atrophy, low vision, and one of the three most common mutations associated with LHON (m.3460G>A, m.11778G>A, or m.l4484T>C) were considered affected. Individuals with the same mutations for LHON as their affected relatives who had no complaints and had no optic atrophy or visual acuity or field deficits on exam were considered unaffected healthy carriers.

Ophthalmologic evaluations: All participants (affected and unaffected) received a detailed clinical examination that included best corrected visual acuity [40,41], pupillary exam, red color saturation, biomicroscopy of the anterior segment, and dilated fundus examinations. Optic nerve photography was done with the Zeiss Digital Imaging System, FF 450 plus (Zeiss FF 450 plus, Carl Zeiss AG, Jena, Germany). In all index cases, we also performed the Goldmann visual field examination test using standard Goldmann perimetry (S-940, Haag-Streit, Bern, Switzerland) and whole brain magnetic resonance imaging (MRI) with contrast (Siemens Symphony, Quantum Gradienten, Maestro class 1.5T, Siemens, Erlangen, Germany).

Molecular genetic analysis: Peripheral blood (5 ml) was collected from affected patients and unaffected relatives and genomic DNA was isolated. Briefly, blood was preserved in EDTA and DNA extraction was completed within 24 h of initial patient blood draw. DNA was isolated from whole blood by a rapid non enzymatic method, involving salting out of the cellular proteins by dehydration and precipitation with saturated sodium chloride solution. DNA was finally precipitated with absolute ethanol and washed with ice-cold 70% ethanol [42]. The mitochondrial DNA was amplified using the technique of chain reaction (PCR) using oligonucleotide pairs specific to the mitochondrial DNA regions where each study mutations: MTND4*LHON11778A (F cccaccttggctatcatc, R

Table 1. Chilean subjects with Leber hereditary optic neuropathy (LHON). Distribution of mutations and haplogroups in affected patients.

Mutation, frequency of participants (n)		Haplogroup	Families (n)	Affected subjects (n)	Age of symptom onset (yrs)
m.11778G>A	59 (78.7%)	A2	1	3	47.3 (±4.9)
		B2i2	2	2	5.5 (±3.5)
		C1b	6	12	17.3 (±8.6)
		L1b	2	3	31.7 (±9.3)
m.14484T>C	12 (16.0%)	B2i2	1	1	34.0
		D1g	1	1	27.0
		J2b	1	2	18.9 (±1.4)
m.3460G>A,	4 (5.3%)	B2i2	1	2	12.0 (±2.8)

Values are mean±standard deviation unless otherwise noted.

ggtaaggcgaggttagcg); MTND1*LHON3460A (F: TTC AAA TTC CTC CCT GTA CG, R: GGC TAC TGC TCG CAG TG). MTND6*LHON14484C (F: ATC CTC CCG AAT CAA CCC TG, R: TTT TTT TAA TTT ATT TAG GGG GCC TG) [43].

Mutational analysis: The presence of one of the three primary LHON mutations was determined with restriction fragment length polymorphism analysis. The amplified fragments were digested with restriction enzymes specific for each mutation: 3460A, Hin1I; NmuCI 11778A, 14484C, MvaI [43] in all affected and unaffected subjects. For all patients, the presence of the specific mutation was confirmed with sequencing. Mitochondrial haplogroup was determined with direct sequencing of the regions HV1 and HV2 and compared with the reference sequences [44].

Pedigree and penetrance calculation: We determined the penetrance of each primary LHON mutation by counting the total number of affected and unaffected siblings of each index patient in each generation. Penetrance was determined by dividing the number of affected by the total per gender.

Data analysis: Statistical analysis was performed using PASW Statistics 18.0.0 (IBM SPSS, New York, NY). Continuous variables were analyzed with ANOVA with post-hoc Bonferroni correction. The Kruskal-Wallis test was used to compare categorical variables between the haplogroups, significance was set at $p<0.05$.

RESULTS

We initially identified 36 unrelated suspected cases. Fifteen (41.7%) of these individuals carried at least one of the three most common mutations associated with LHON. After obtaining informed consent, we examined 75 affected and unaffected carriers in the 15 families.

Of 26 affected cases, 21 (80.7%) were men, and five (19.3%) were women. In carrier subjects, 36 (73.5%) were women, and 13 (26.5%) were men. The overall penetrance observed in this study was 61.8% (21 of 34) for the male and 12.2% (five of 36) for the female subjects. The differences in penetrance of the different haplogroups could not be effectively analyzed because of the small size of the groups. The gender ratio in the studied population was 4.2/1 with 21 men and five women affected, and 13 carrier men and 36 carrier women (Table 1).

The mean age of onset of symptoms was 22.2±13.4 years old, ranging between 3 and 53 years. Five of the affected began to note symptoms before age 10, with two of these before 6 years of age; both were female (Table 2).

In the affected subjects, the mean time between symptom onset and first examination was 7.8±4.9 days (range 12 h and 21 days). Six patients (23.1%) reported that visual acuity loss was simultaneous in both eyes. In two patients (7.7%), the age of vision loss could not be determined because it happened early in childhood. The remainder (69.2%) reported sequential decrease in acuity. In patients with sequential symptoms, the mean time to involvement of the second eye was 35.1 days (range 7 and 270 days).

The time to visual acuity stabilization was highly variable, with a mean of 7.8±8.1 months in those whose vision loss stabilized during the study time period. Eleven patients (42.3%) reported exposure to tobacco, alcohol, or drugs before the onset of symptoms. We did not find a statistically significant relationship between the consumption of alcohol, tobacco, and drugs and the manifestation of the disease in the sample of patients studied.

Of the 75 subjects with one of the three common mutations, 59 subjects (78.7%) had the m.11778G>A mutation, 12

Table 2. Phenotypic features of twenty-six patients from the fifteen families in Chile affected by Leber Hereditary Optic Neuropathy.

Mutation	Patient	Family	Haplogroup	Gender	Age at exam (years)	Age of onset (years)	Best corrected acuity (right/ left eye, fractional)
m.11778G>A	1	2	A2	M	58	53	CF/CF
	2	2	A2	M	54	44	CF/CF
	3	2	A2	M	56	45	CF/CF
	4	6	B2i2	F	6	3	CF/0.05
	7	12	B2i2	M	43	8	CF/CF
	9	3	C1b	M	27	24	CF/CF
	10	3	C1b	M	35	8	0.3/0.3
	11	3	C1b	M	46	17	0.3/0.3
	12	4	C1b	M	22	19	CF/CF
	13	4	C1b	F	56	5	0.1/0.1
	14	5	C1b	M	22	20	0.05/CF
	15	5	C1b	F	38	36	CF/CF
	16	9	C1b	M	19	17	CF/CF
	17	9	C1b	M	24	22	HM/HM
	18	11	C1b	M	23	20	0.05/0.05
	19	11	C1b	M	8	6	CF/CF
	20	15	C1b	M	16	14	CF/0.05
	24	7	L1b	M	66	29	HM/HM
	25	7	L1b	F	64	42	LP/HM
	26	14	L1b	M	44	24	CF/CF
m.14484T>C	8	10	B2i2	M	36	34	0.05/0.05
	21	13	D1g	M	29	27	0.05/0.15
	22	1	J2b	M	30	19	0.1/0.05
	23	1	J2b	M	37	17	0.05/0.1
m.3460G>A	5	8	B2i2	F	38	14	0.05/0.05
	6	8	B2i2	M	12	10	0.05/0.05

The following abbreviations were used; male (M), female (F), CF=counting fingers, HM=hand motions and LP=light perception.

subjects (16.0%) had the m.14484T>C mutation, and four subjects (5.3%) had the m.3460G>A mutation in this population (Table 1).

Twelve families had Amerindian haplogroups: One family had the A2 haplogroup, four families had the B2i2 haplogroup, six families had the Cb haplogroup, and one family had the D1g haplogroup. One family had the J2b haplogroup, which is associated with European populations, and two families had the L1b haplogroup, which is associated with African populations (Table 2).

Of the affected subjects, 92.3% had fractional visual acuity equal to or less than 0.1 (≥1.0 logMar) in the better eye. Only in two subjects of family 3 (m.11778G>A mutation and haplogroup C1b) did we observe fractional visual acuity better than 0.1 in the better eye.

In 83% of the affected patients, symmetric constriction of the pupil and no relative afferent pupillary defect were noted; in 17% of the affected patients, the pupillary light reflexes were present but remarkably slower. The visual field was altered in all patients, with central scotomas of varying intensity and size and cecocentral scotomas with concentric restriction of isopters.

Subjects with haplogroup C1b were more likely to retain 20/200 or better vision in the better eye than those with other haplogroups (p=0.033, Kruskal–Wallis). Subjects with the A2 haplogroup on average had later onset of disease (p=0.001,

ANOVA) than those with other haplogroups (47.3 years on average).

DISCUSSION

Molecular testing for LHON is not routinely performed in patients with optic atrophy in Chile. The 36 patients who were initially identified as candidates for molecular testing had undergone complete clinical testing performed by local neuroophthalmology departments and had been suspected of having LHON. We believe that the high pretest probability led to the high percentage of patients with a positive molecular test result. We did not further evaluate those with a negative molecular test for either the less frequent mutations associated with LHON or other genetic causes of optic atrophy.

Few studies have evaluated Amerindian mDNA haplogroups, either Central American or South American [26,27,32]. Studies of mDNAs in native Chilean populations (Mapuche, Pehuenche, and Yaghans), with a mixture of different ethnic groups and populations, have shown the following distribution by restriction fragment length polymorphism analysis and D-loop (control region) sequencing: 1.3% had haplogroup A, 8% haplogroup B, 43% haplogroup C, and 47.7% haplogroup D [26]. Another study of individuals from the admixed population of Santiago found that 8.1% had haplogroup A, 29% haplogroup B, 37.9% haplogroup C, and 25% haplogroup D [27].

The number of cases in the study is small but given current prevalence estimates likely accounts for around 10% of the cases in Chile given the current population of 16.6 million [1,3]. Due to the limited sample size, we are unable to make a formal assessment of the effect of haplogroup on penetrance and onset of disease. However, despite the relative lack of information regarding the overall accuracy of the described mDNA composition of the population in Chile, some authors suggest that Chilean populations are dominated by haplogroups C and D [26]. It is remarkable that in our study only one family out of 15 had haplogroup D, as haplogroup D is one of the most common in the Chilean population, with a frequency of 25% to 40% [26] and is the most prevalent in larger population samples from the Southern Cone of South America (Chile and Argentina) [32]. This could be a result of the relatively small population studied, but we cannot rule out that haplogroup D has a protective effect in carriers of LHON mutations similar to what has been noted with haplogroup H in European populations.

The penetrance across all families in this study was 61.8% for men and 12.2% for women. The gender ratio in this population was 4.2/1 with 21 men and five women affected, and 13 carrier men and 36 carrier women. The incomplete general penetrance and the gender ratio in this population are similar to those reported for other LHON populations around the world, with approximately 50% of men and approximately 10% of women affected [2,45-50].

We also found that 92.3% of affected subjects had fractional visual acuity equal or less than 0.1. We did not observe any cases in which there was a meaningful recovery of vision, as reported by other authors [2]. In addition, no differences were observed in final visual acuity among the three mutations, which again may have been due to the relatively small number of patients.

In summary, haplogroup is one genetic factor suspected of modulating LHON. Recent studies suggest that haplogroups play an important role in the onset of LHON, and in particular, haplogroup and phylogenetic analyses suggest that one European-specific mDNA background plays a role in the expression of LHON by increasing the clinical penetrance of the primary mutations m.11778G>A and m.14484T>C. Because of the continent-specific distribution of mDNA haplogroups, there is a remarkable difference in these haplogroups, which may influence the expression of LHON in different ethnic groups. We find that there was a markedly decreased frequency of haplogroup D in Chilean subjects with LHON compared to what has been reported for the overall population. Future studies of populations with similar haplogroups could assist in providing support to the present findings and provide new clues on the molecular mechanism by which a particular mDNA background could influence clinical expression of LHON.

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REFERENCES

1. Man PY, Griffiths PG, Brown DT, Howell N, Turnbull DM, Chinnery PF. The epidemiology of Leber hereditary optic neuropathy in the North East of England Am J Hum Genet 2003; 72:333-9. [PMID: 12518276].

2. Spruijt L, Kolbach DN, de Coo RF, Plomp AS, Bauer NJ, Smeets HJ, de Die-Smulders CE. Influence of mutation type

on clinical expression of Leber hereditary optic neuropathy. Am J Ophthalmol 2006; 141:676-82. [PMID: 16564802].

3. Puomila A, Hämäläinen P, Kivioja S, Savontaus ML, Koivumäki S, Huoponen K, Nikoskelainen E. Epidemiology and penetrance of Leber hereditary optic neuropathy in Finland. Eur J Hum Genet 2007; 15:1079-89. [PMID: 17406640].
4. Yu D, Jia X, Zhang AM, Li S, Zou Y, Zhang Q, Yao YG. Mitochondrial DNA sequence variation and haplogroup distribution in Chinese patients with LHON and m.14484T>C. PLoS ONE 2010; 5:e13426-[PMID: 20976138].
5. Bi R, Zhang AM, Yu D, Chen D, Yao YG. Screening the three LHON primary mutations in the general Chinese population by using an optimized multiplex allele-specific PCR. Clin Chim Acta 2010; 411:1671-4. [PMID: 20599858].
6. Harding AE, Sweeney MG, Govan GG, Riordan-Eva P. Pedigree analysis in Leber hereditary optic neuropathy families with a pathogenic mtDNA mutation. Am J Hum Genet 1995; 57:77-86. [PMID: 7611298].
7. Yu-Wai-Man P, Griffiths PG, Hudson G, Chinnery PF. Inherited mitochondrial optic neuropathies. J Med Genet 2009; 46:145-58. [PMID: 19001017].
8. Yen MY, Wang AG, Wei YH. Leber's hereditary optic neuropathy: a multifactorial disease. Prog Retin Eye Res 2006; 25:381-96. [PMID: 16829155].
9. Carelli V, Ross-Cisneros FN, Sadun AA. Mitochondrial dysfunction as a cause of optic neuropathies. Prog Retin Eye Res 2004; 23:53-89. [PMID: 14766317].
10. Man PY, Turnbull DM, Chinnery PF. Leber hereditary optic neuropathy. J Med Genet 2002; 39:162-9. [PMID: 11897814].
11. Wong LJ. Pathogenic mitochondrial DNA mutations in protein-coding genes. Muscle Nerve 2007; 36:279-93. [PMID: 17503499].
12. Mitomap; http://www.mitomap.org
13. Torroni A, Petrozzi M, D'Urbano L, Sellitto D, Zeviani M, Carrara F, Carducci C, Leuzzi V, Carelli V, Barboni P, De Negri A, Scozzari R. Haplotype and phylogenetic analyses suggest that one European-specific mtDNA background plays a role in the expression of Leber hereditary optic neuropathy by increasing the penetrance of the primary mutations 11778 and 14484. Am J Hum Genet 1997; 60:1107-21. [PMID: 9150158].
14. Hudson G, Carelli V, Spruijt L, Gerards M, Mowbray C, Achilli A, Pyle A, Elson J, Howell N, La Morgia C, Valentino ML, Huoponen K, Savontaus ML, Nikoskelainen E, Sadun AA, Salomao SR, Belfort R Jr, Griffiths P, Man PY, de Coo RF, Horvath R, Zeviani M, Smeets HJ, Torroni A, Chinnery PF. Clinical expression of Leber hereditary optic neuropathy is affected by the mitochondrial DNA-haplogroup background. Am J Hum Genet 2007; 81:228-33. [PMID: 17668373].
15. Kerrison JB, Miller NR, Hsu F, Beaty TH, Maumenee IH, Smith KH, Savino PJ, Stone EM, Newman NJ. A case-control study of tobacco and alcohol consumption in Leber hereditary optic neuropathy. Am J Ophthalmol 2000; 130:803-12. [PMID: 11124301].
16. Hudson G, Keers S, Yu Wai Man P, Griffiths P, Huoponen K, Savontaus ML, Nikoskelainen E, Zeviani M, Carrara F, Horvath R, Karcagi V, Spruijt L, de Coo IF, Smeets HJ, Chinnery PF. Identification of an X-chromosomal locus and haplotype modulating the phenotype of a mitochondrial DNA disorder. Am J Hum Genet 2005; 77:1086-91. [PMID: 16380918].
17. Howell N, Herrnstadt C, Shults C, Mackey DA. Low penetrance of the 14484 LHON mutation when it arises in a non-haplogroup J mtDNA background. Am J Med Genet A 2003; 119A:147-51. [PMID: 12749053].
18. Ghelli A, Porcelli AM, Zanna C, Vidoni S, Mattioli S, Barbieri A, Iommarini L, Pala M, Achilli A, Torroni A, Rugolo M, Carelli V. The background of mitochondrial DNA haplogroup J increases the sensitivity of Leber's hereditary optic neuropathy cells to 2,5-hexanedione toxicity. PLoS ONE 2009; 4:e7922-[PMID: 19936068].
19. Cooper JM. Vol. 2, pp 687–760. Washington D.C, United State: The Araucanians, Handbook of South American Indians, Smithsonian Institute, Bureau of American Ethnology; 1946.
20. Bengoa J. Chapters 1–3. Santiago, Chile: Historia del Pueblo Mapuche (Siglos XIX y XX); 1985.
21. Mellafe R. Chapter 5. Santiago, Chile: La introducción de la esclavitud negra en Chile. Tráfico y rutas; 1959.
22. Gonzalo Vial Correa. El africano en el Reino de Chile. Ensayo histórico-jurídico. Santiago, Chile 1957.
23. Cruz GF. Santiago, Chile: La abolición de la esclavitud en Chile; 1973.
24. Carlos Valenzuela Y. El Gradiente Sociogenético Chileno y sus Implicaciones Etico-Sociales. Santiago, Chile 2002.
25. Bailliet G, Rothhammer F, Carnese FR, Bravi CM, Bianchi NO. Founder mitochondrial haplotypes in Amerindian populations. Am J Hum Genet 1994; 55:27-33. [PMID: 7517626].
26. Moraga ML, Rocco P, Miquel JF, Nervi F, Llop E, Chakraborty R, Rothhammer F, Carvallo P. Mitochondrial DNA polymorphisms in Chilean aboriginal populations: implications for the peopling of the southern cone of the continent. Am J Phys Anthropol 2000; 113:19-29. [PMID: 10954617].
27. Rocco P, Morales C, Moraga M, Miquel JF, Nervi F, Llop E, Carvallo P, Rothhammer F. Genetic composition of the Chilean population. Analysis of mitochondrial DNA polymorphism. Rev Med Chil 2002; 130:125-31. [PMID: 11974524].
28. Achilli A, Perego UA, Bravi CM, Coble MD, Kong QP, Woodward SR, Salas A, Torroni A, Bandelt HJ. The Phylogeny of the Four Pan-American MtDNA Haplogroups: Implications for Evolutionary and Disease Studies. PLoS ONE 2008; 3:e1764-[PMID: 18335039].
29. Llop E. Genetic composition of Chilean aboriginal populations: HLA and other genetic marker variation. Am J Phys Anthropol 1996; 101:325-32. [PMID: 8922179].

30. Llop E, Harb Z, Acuña M, Moreno R, Barton S, Aspillaga E, Rothhammer F. Genetic composition of Chilean population: the Pehuenches from Trapa-Trapa. Rev Med Chil 1993; 121:494-8. [PMID: 8272628].

31. Llop E, Harb Z, Moreno R, Aspillaga E, van de Maele M, Rothhammer F. Genetic composition of the Chilean population: the Yamanas from Ukika. Rev Med Chil 1994; 122:979-85. [PMID: 7597344].

32. Bodner M, Perego UA, Huber G, Fendt L, Röck AW, Zimmermann B, Olivieri A, Gómez-Carballa A, Lancioni H, Angerhofer N, Bobillo MC, Corach D, Woodward SR, Salas A, Achilli A, Torroni A, Bandelt HJ, Parson W. Rapid coastal spread of First Americans: novel insights from South America's Southern Cone mitochondrial genomes. Genome Res 2012; 22:811-20. [PMID: 22333566].

33. Kaewsutthi S, Phasukkijwatana N, Joyjinda Y, Chuenkongkaew W, Kunhapan B, Tun AW, Suktitipat B, Lertrit P. Mitochondrial haplogroup background may influence Southeast Asian G11778A Leber hereditary optic neuropathy. Invest Ophthalmol Vis Sci 2011; 52:4742-8. [PMID: 21398275].

34. Ji Y, Zhang AM, Jia X, Zhang YP, Xiao X, Li S, Guo X, Bandelt HJ, Zhang Q, Yao YG. Mitochondrial DNA haplogroups M7b1'2 and M8a affect clinical expression of leber hereditary optic neuropathy in Chinese families with the m.11778G→a mutation. Am J Hum Genet 2008; 83:760-8. [PMID: 19026397].

35. Zhang AM, Jia X, Bi R, Salas A, Li S, Xiao X, Wang P, Guo X, Kong QP, Zhang Q, Yao YG. Mitochondrial DNA haplogroup background affects LHON, but not suspected LHON, in Chinese patients. PLoS ONE 2011; 6:e27750-[PMID: 22110754].

36. Sadun AA, Carelli V, Salomao SR, Berezovsky A, Quiros PA, Sadun F, DeNegri AM, Andrade R, Moraes M, Passos A, Kjaer P, Pereira J, Valentino ML, Schein S, Belfort R. Extensive investigation of a large Brazilian pedigree of 11778/haplogroup J Leber hereditary optic neuropathy. Am J Ophthalmol 2003; 136:231-8. [PMID: 12888043].

37. Sadun AA, Carelli V, Salomao SR, Berezovsky A, Quiros P, Sadun F, DeNegri AM, Andrade R, Schein S, Belfort R. A very large Brazilian pedigree with 11778 Leber's hereditary optic neuropathy. Trans Am Ophthalmol Soc 2002; 100:169-78. , discussion 178–9.. [PMID: 12545691].

38. Maciel-Guerra AT, Zanchetta LM, Amaral Fernandes MS, Andrade PB, do Amor Divino Miranda PM, Sartorato EL. Leber's hereditary optic neuropathy: clinical and molecular profile of a Brazilian sample. Ophthalmic Genet 2010; 31:126-8. [PMID: 20565249].

39. Sanchez RN, Smith AJ, Carelli V, Sadun AA, Keltner JL. Leber hereditary optic neuropathy possibly triggered by exposure to tire fire. J Neuroophthalmol 2006; 26:268-72. [PMID: 17204920].

40. Bailey IL, Lovie JE. New design principles for visual acuity letter charts. Am J Optom Physiol Opt 1976; 53:740-5. [PMID: 998716].

41. Lange C, Feltgen N, Junker B, Schulze-Bonsel K, Bach M. Resolving the clinical acuity categories "hand motion" and "counting fingers" using the Freiburg Visual Acuity Test (FrACT). Graefes Arch Clin Exp Ophthalmol 2009; 247:137-42. [PMID: 18766368].

42. Lahiri DK, Nurnberger JI Jr. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. Nucleic Acids Res 1991; 19:5444-[PMID: 1681511].

43. Brown MD, Torroni A, Reckord CL, Wallace DC. Phylogenetic analysis of Leber's hereditary optic neuropathy mitochondrial DNA's indicates multiple independent occurrences of the common mutations. Hum Mutat 1995; 6:311-25. [PMID: 8680405].

44. Andrews RM, Kubacka I, Chinnery PF, Lightowlers RN, Turnbull DM, Howell N. Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. Nat Genet 1999; 23:147-[PMID: 10508508].

45. Seedorff T. The inheritance of Leber's disease. A genealogical follow-up study. Acta Ophthalmol (Copenh) 1985; 63:135-45. [PMID: 4003041].

46. Newman NJ. Hereditary optic neuropathies: from the mitochondria to the optic nerve. Am J Ophthalmol 2005; 140:517-23. Review[PMID: 16083845].

47. Riordan-Eva P, Sanders MD, Govan GG, Sweeney MG, Da Costa J, Harding AE. The clinical features of Leber's hereditary optic neuropathy defined by the presence of a pathogenic mitochondrial DNA mutation.xxxxxx 1995;xx:319–37.

48. Macmillan C, Kirkham T, Fu K, Allison V, Andermann E, Chitayat D, Fortier D, Gans M, Hare H, Quercia N, Zackon D, Shoubridge EA. Pedigree analysis of French Canadian families with T14484C Leber's hereditary optic neuropathy. Neurology 1998; 50:417-22. [PMID: 9484365].

49. Newman NJ. Leber's hereditary optic neuropathy. New genetic considerations. Arch Neurol 1993; 50:540-8. [PMID: 8489411].

50. Johns DR, Smith KH, Miller NR. Leber's hereditary optic neuropathy. Clinical manifestations of the 3460 mutation. Arch Ophthalmol 1992; 110:1577-81. [PMID: 1444915].

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