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Report

A "*Fille du Roy*" Introduced the T14484C Leber Hereditary Optic Neuropathy Mutation in French Canadians

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The predominance of the T14484C mutation in French Canadians with Leber hereditary optic neuropathy is due to a founder effect. By use of genealogical reconstructions of maternal lineages, a woman married in Quebec City in 1669 is identified as the shared female ancestor for 11 of 13 affected individuals, who were previously not known to be related. These individuals carry identical mitochondrial haplogroups. The current geographic distribution of French Canadian cases overlaps with that of the founder's female descendants in 1800. This is the first example of genealogical reconstruction to identify the introduction of a mitochondrial mutation by a woman in a founder population.

Leber hereditary optic neuropathy (LHON [MIM 535000]) presents as acute or subacute loss of central vision in an individual with previously normal vision (Man et al. 2002). In most cases, both eyes are affected, either simultaneously or sequentially. In some cases, vision eventually improves. LHON is maternally transmitted and affects primarily young adult males. In >95% of cases, LHON is caused by one of three mutations—G3640A, G11778A, or T14484C—located in the mitochondrial genes encoding respiratory chain complex I subunits. G11778A is the most prevalent mutation worldwide (52%–92% of cases), T14484C is usually the second most prevalent (3%–19% of cases), and G3640A is the least prevalent (1%–33% of cases) (Rosenberg et al. 1995; Yen et al. 2002; Howell et al. 2003; Kim et al. 2003; Man et al. 2003). In contrast, the most frequently found mutation in French Canadian patients is T14484C, which accounts for 86% of French Canadians cases, whereas G11778A and G3640A account for 12% and 2%, respectively (Macmillan et al. 1998). Haplogroup analysis suggested that the predominance of the T14484C mutation in French Canadian patients with LHON was due to a founder effect and that those patients might all descend from the same woman (Macmillan et al. 2000). Dutch patients with LHON who carry the T14484C mutation share the same mitochondrial haplogroup, which suggests that this mutation appeared in Europe earlier than the first French settlement in Quebec in 1608 (Howell et al. 2003). The first report of LHON in French Canadians dates to 1970, when a large pedigree from southwestern Quebec was described (Brunette and Bernier 1970). A high rate of spontaneous recovery was observed in this large family.

Through use of Quebec's genealogical records, we wanted to determine if a single female founder introduced the LHON T14484C mutation in French Canadians. In the province of Quebec, LHON mitochondrial genotyping is performed in a single laboratory at the Montreal Neurological Institute (E.A.S., unpublished data). Twenty-four physicians who sent samples for

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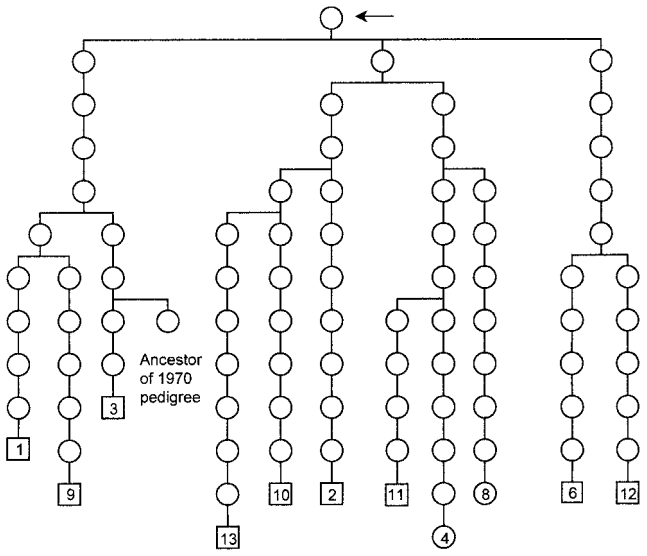

Figure 1 Pedigree of the 11 probands sharing the same female ancestor and their relationship with the family reported in 1970 (Brunette and Bernier 1970).

LHON genotyping were asked to contact their patients with T14484C LHON and inquire whether the patients would agree to be contacted by our team. Patients who agreed to participate in the study returned a written consent form after having been informed of the nature of the project by a research nurse. Each participant provided the following information required for maternal lineage reconstruction: name, date of birth, and dates and parishes of marriages of mother and maternal grandmother. This information was used to construct direct maternal lineages for each individual all the way to the female ancestor who settled in Nouvelle-France. This was done by Project BALSAC by use of the RETRO databases (Bouchard 2004). Ascending maternal lineage genealogies were obtained for 13 unrelated individuals with T14484C LHON, thereafter called "probands." Twelve were successfully reconstructed (probands 1–4 and 6–13). Eleven were found to be related to the same female founder through maternal lineage (probands 1–4, 6, and 8–13) (fig. 1). One of the authors of the original 1970 report provided us with the name of the oldest obligate-carrier female ancestor identified in their pedigree, who married before 1870 (R. G. Bernier, personal communication). As shown in figure 1, that carrier ancestor is related through her maternal lineage to the same female founder.

This female founder was born in France. She came to Nouvelle-France as a "*fille du roy*" and married in Quebec City in 1669. The 737 "*filles du roy,*" or "the King's daughters," were women sent by King Louis XIV, between 1663 and 1673, to Nouvelle-France to marry (Charbonneau et al. 1987), as an attempt to correct the unfavorable male:female ratio in the young colony and thereby encourage the permanent settlement of immigrants in Nouvelle-France. The ratio went from 169 males per 100 females in 1608–1662 to 118 males per 100 females in 1663–1679 (Charbonneau et al. 1987). While still in France, these young women had been placed under the care of the king because they were orphans or single women without sufficient family support.

Statistical analysis was performed to estimate the probability that the identification of a single female founder shared by 11 individuals through maternal lineage could be due to chance. By use of the maternal lineages of 2,600 available existing genealogies in the RETRO database of the Project BALSAC, 5,000 simulations of reconstruction of maternal lineages were performed. Each simulation used 11 subjects selected from among the starting individuals of the 2,600 ascending genealogies. Each individual was matched with one of the probands with LHON for the region of his or her parents' wedding. This was done to control for differences in genetic characteristics of the different regional populations. In none of the 5,000 simulations was a single female founder ($f = 1$) shared by all of the 11 randomly selected individuals. Since the probability of finding less than seven shared female founders related only through maternal lineages ($f < 7$) by chance for 11 control individuals is <0.002 (table 1), these results strongly support the identification of this single female founder as the actual source of the main introduction of the T14484C mutation in the French Canadian population.

Further evidence to support this conclusion comes from the complete sequencing of the HV1 and HV2 hypervariable mtDNA regions in a subset of these patients.

Table 1

Probability of Finding *f* Founder Females by Use of 5,000 Simulations of the Maternal-Lineage Pedigree Reconstructions of 11 Random Probands

No. of Founder Females Identified (*f*)	No. of Simulations (*n*) in Which *f* Founder Females Were Identified (of 5,000 total)	Probability of Finding *f* Founder Females (*n*/5,000)
1	0	0
2	0	0
3	0	0
4	0	0
5	0	0
6	0	0
7	1	.0002
8	28	.0056
9	246	.0492
10	1,426	.2852
11	3,299	.6598

NOTE.—The 11 probands were matched with original probands for location of their parents' weddings.

Sequencing was performed on PCR products as done by Macmillan et al. (1998), by use of an ABI 3730 XL sequencer for 9 of the 11 probands related to the founder; for proband 5, who was found not to be related to her; for proband 7, for whom genealogical reconstruction was not possible; and for 21 other French Canadian individuals with LHON, who were previously thought to be unrelated. Table 2 shows that all nine related carriers share the same haplogroup, as do proband 7 and the 21 other unrelated French Canadians with LHON. The rare differences observed likely represent spontaneous mutations in the founder haplogroup. Proband 5 clearly does not share the same haplogroup, which is consistent with the genealogical results that he inherited the T14484C mutation through a different maternal lineage (table 2).

Since LHON is a rare disease in Quebec, we wanted to assess this founder's contribution to the French Canadian gene pool. The genetic contribution (GC) of an ancestor to an individual is inversely proportional to the number of generations between the ancestor and that individual through a specific genealogical link (g) and proportional to the number of genealogical links between the two (c):

$$\mathrm{GC} = \sum_{i=1}^{c} (1/2)^{g_i} \ .$$

Total genetic contribution of an ancestor to a group of individuals is the proportion of the genetic pool of said group that comes from said ancestor. It is the sum of the genetic contributions of the ancestor to each individual,

$$\mathrm{GC}_{\mathrm{tot}} = \sum_{j=1}^{n} \sum_{i=1}^{c} (1/2)^{g_{i,j}} \ ,$$

where n denotes the number of individuals in the group (Gagnon and Heyer 2001). This measures the contribution of an individual to the autosomal gene pool, not the mitochondrial gene pool, but we have used it as a proxy for overall genetic contribution to the French Canadian population. With currently available databases, no adequate method is available to assess the relative contribution of female founders to the Quebec mitochondrial gene pool. The total genetic contribution of the female founder in question to the French Canadian population of Quebec is estimated at the 30th percentile. This lower-than-average genetic contribution is consistent with the relatively low prevalence of LHON in Quebec.

The availability of a complete computerized database of all Quebec civil records up to the year 1800 allowed us to assess whether early migratory events shaped the present distribution of T14484C LHON cases in Quebec. Using the PRDH (Programme de Recherche en Demographie Historique) database (Charbonneau et al. 1987), a descending genealogy from 1669 to 1800 was reconstructed, starting with the female founder presumed to introduce the mutation (maximum of 5 generations, total of 49 female descendants through females only). The parishes of weddings were used to geographically map the migration of her female descendants (fig. 2). The founder female's five daughters were all married in or near Quebec City, but only half of her great-granddaughters were married there. Eleven of her 21 great-granddaughters and 10 of her 13 great-great-granddaughters were married in the greater Montreal region (incomplete data for the 5th generation; data available only until 1800). This predominant migration of her female descendants to the southwestern part of what is now the province of Quebec corresponds well with the present geographical distribution of T14484C LHON cases. Postal codes from 45 Quebec patients with T14484C show that 40

Table 2

Haplogroup Analysis of French Canadians with LHON

	Haplotype at Site of Hypervariable mtDNA Region																		
	HV2										HV1								
Analysis of	73	152	185	199	204	207	228	250	263	295	16069	16126	16129	16189	16213	16223	16260	16261	16292
Human mitochondrial genome[a]	A	T	G	T	T	G	G	T	A	C	C	T	G	T	G	C	C	C	C
Consensus[b]	G	T	A	T	T	G	A	T	G	T	T	C	G	T	A	C	C	C	C
Probands 2–4, 6, 7, and 9–12	G	T	A	T	T	G	A	T	G	T	T	C	G	T	A	C	C	C	C
Proband 8	ND	T	A	T	T	G	A	T	G	T	T	C	G	T	A	C	C	C	C
Proband 5	G	C	G	C	A	A	G	C	G	C	C	T	A	C	G	T	C	T	C
Individual A	G	T	A	T	T	G	A	T	G	T	T	C	G	T	A	C	C/T	C	C
Individuals B–H and J–U	G	T	A	T	T	G	A	T	G	T	T	C	G	T	A	C	C	C	C
Individual I	G	T	A	T	T	G	A	T	G	T	T	C	G	T	G	C	C	C	T
Individual V	ND	T	ND	T	T	G	A	T	G	T	T	C	G	T	A	C	C	C	C

Note.—ND = not done.

* As found in GenBank.

[b] Consensus sequence in French Canadian T14484C carriers.

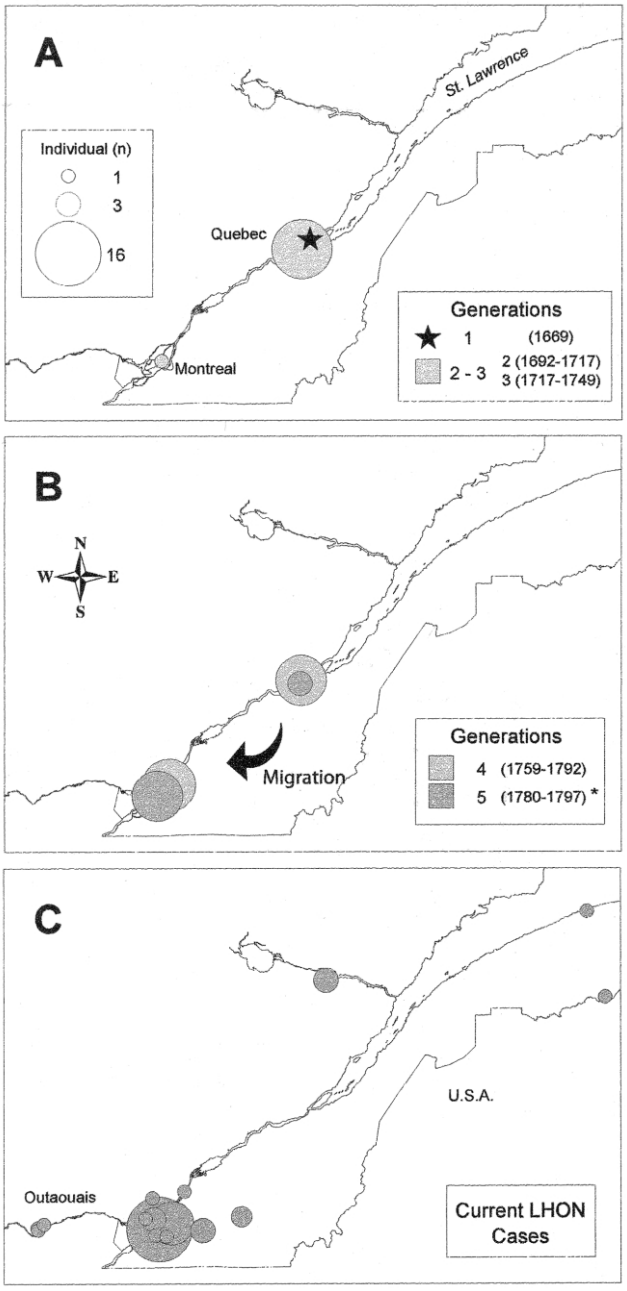

Figure 2 Geographic distribution of the marriage parishes of the single female ancestor's female descendants, by generation (1669–1800) (*A* and *B*) and geographic distribution of current cases that are due to T14484C mutation (*C*). An asterisk (*) marks the 5th generation, which is incomplete, because data are available only through 1800.

live in southwestern Quebec. Another 16 patients live in Ontario, mainly near the southwestern border of Quebec.

The use of the PRDH database also allowed us to assess whether males with LHON in the early colony had more difficulty finding spouses. This hypothesis was tested using male descendants of the single common female ancestor, born before 1800 and related through females only. Of the 45 descendants, 17 (38%) were married, compared with 13 (30%) of the 43 male controls, matched for year of birth ($\chi^2 = 0.557$; $P = .455$). Although the small numbers involved limit our ability to conclude from these results, these results are not suggestive of a detrimental effect of LHON on ability to find a spouse in the first generations of male descendants. This could be explained either by the occurrence of symptoms after the age of marriage or by the high rate of spontaneous recovery in patients with the T14484C mutation.

By combining genealogical and molecular data sets, we have established that the French Canadian founder effect for the predominant LHON T14484C mutation in Quebec was most likely due to a 17th century introduction of this mutation by a female founder. This said, there was more than one introduction of this mutation into this population, as demonstrated by proband 5. The study of French Canadian computerized genealogies can allow the identification of pioneers who introduced mutations into this population. To our knowledge, this is the first published report of the identification of an introducer of a mitochondrial mutation in a founder population. Early migrational events have shaped the current differences in regional prevalence of mutations in Quebec.

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Web Resources

The URLs for data presented herein are as follows:

GenBank, http://www.ncbi.nlm.nih.gov/Genbank/ (for the human mitochondrial genome)
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for LHON)
Projet BALSAC, http://www.uqac.uquebec.ca/balsac/
PRDH, http://www.genealogie.umontreal.ca/fr/

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