# Letters to the Editor

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## Detection of the mtDNA 14484 Mutation on an African-Specific Haplotype: Implications about Its Role in Causing Leber Hereditary Optic Neuropathy

*To the Editor:*

Leber hereditary optic neuropathy (LHON) is a maternally transmitted disease whose primary clinical manifestation is acute or subacute bilateral loss of central vision leading to central scotoma and blindness. To date, LHON has been associated with 18 mtDNA missense mutations (Wallace et al. 1995), even though, for many of these mutations, it remains unclear whether they cause the disease, contribute to the pathology, or are nonpathogenic mtDNA polymorphisms (Riordan-Eva and Harding 1995). On the basis of numerous criteria, which include the specificity for LHON, the frequency in the general population, and the penetrance within affected pedigrees, the detection of associated defects in the respiratory chain, mutations at three nucleotide positions (nps), 11778 (G→A) (Wallace et al. 1988), 3460 (G→A) (Houponen et al. 1991; Howell et al. 1991), and 14484 (T→C) (Johns et al. 1992, 1993; Mackey and Howell 1992) have been classified as high-risk and primary LHON mutations (Newman 1993; Brown and Wallace 1994). Overall, these three mutations encompass ⩾90% of the LHON cases (Savontaus 1995).

From an evolutionary point of view, there is a major criterion that helps to distinguish severe mtDNA mutations from low-risk mtDNA mutations and/or population-specific polymorphisms. High-risk mutations, i.e., mutations that do not require additional mtDNA mutations to cause the phenotype and have a high degree of penetrance, are under a relatively strong selective pressure. Therefore, they are likely to be lost, together with the mtDNAs on which they occurred, within few generations from their occurrence. However, these mutations are continuously generated in the general population and continuously generate new affected pedigrees. Since the new mutational events occur randomly, severe mtDNA mutations are expected to be found on heterogeneous mtDNA backgrounds and in all human populations. On the contrary, low-risk mutations with lower penetrance are under a milder selective pressure and, similar to neutral mutations, can be transmitted for numerous generations. Transmission through numerous generations makes it possible for a mutation generated by a single mutational event to be transmitted by descent to numerous pedigrees, which would appear to be unrelated even though they share a female ancestor. If most of the pedigrees affected are thus related, these mutations may appear as population specific.

The LHON mutations at nps 11778 and 3460 fit the expectations for severe mtDNA mutations. The 11778 mutation represents 50%–90% of the LHON cases in Europeans (Newman 1993) and 80%–90% of cases in Japan (Mashima et al. 1993). Haplotype analyses have shown that this mutation occurred on a wide range of mtDNA backgrounds that include European and African-specific haplogroups (Newman et al. 1994; Brown et al. 1995). The 3460 mutation represents 8%–25% of European LHON cases (Savontaus 1995) and 3%–4% of Japanese (Ishikawa et al. 1995). The 3460 mutation has appeared on all common European mtDNA haplogroups in North Americans (Brown et al. 1995).

The 14484 mutation, the third "severe" LHON mutation, has been found in European (~10%) (Savontaus 1995) and Japanese (~3%–4%) (Ishikawa et al. 1995) LHON patients. However, its primary role in causing LHON is less defined. In contrast to the 11778 and 3460 mutations, which are often heteroplasmic, the 14484 mutation is usually homoplasmic. Rare heteroplasmic cases have been found only in association with other

primary mutations (Mackey and Howell 1992; Harding et al. 1995), thus suggesting that heteroplasmy of the 14484 mutation is not sufficient to cause the disease. The mutation does not affect complex I activity (Cock et al. 1995), and affected LHON subjects have a high rate of partial visual recovery, particularly if the age at onset is <20 years (Johns et al. 1993; Riordan-Eva et al. 1995). Haplotype analyses of mtDNAs from Caucasian patients from North America have shown that 70%–75% of the LHON pedigrees with the 14484 mutation harbor mtDNAs belonging to haplogroup J (Johns et al. 1993; Brown et al. 1995), one of the European-specific mtDNA haplogroups (Torroni et al. 1994*b*). This association has also been observed in Australian LHON families of European ancestry (Howell et al. 1995). Haplogroup J encompasses 9.0% of mtDNAs in the general Caucasian population from North America (Torroni et al. 1994*b*), so the incidence of the 14484 mutation on this haplogroup is almost eightfold higher than it is expected if the 14484 mutation occurs on all mtDNA backgrounds randomly. All haplogroup J mtDNAs are characterized by the presence of the two putative "secondary" LHON mutations, those at nps 13708 and 4216 (Torroni et al. 1994*b;* Brown et al. 1995; Howell et al. 1995). These observations raise the possibility that the 14484 requires additional mtDNA mutations to cause LHON and thus is not a primary LHON mutation.

We now report a LHON patient from Tunisia whose mtDNA was found to harbor the 14484 mutation in the absence of "secondary" mutations and to belong to an African-specific mtDNA haplogroup. These data suggest that the 14484 mutation is sufficient to cause LHON. The patient is a 25-year-old Tunisian male living in Italy who experienced progressive and painless blurring of central visual acuity in his left eye, with similar involvement of the second eye after 15 d. A bilateral, large central scotoma was evident at visual field examination, and swelling of the optic disc and peripapillary microangiopathy were revealed by fluorescein angiogram and ophthalmoscopic examination in both eyes. The LHON diagnosis was also supported by anamnestic report from the proband, which indicated that similar visual problems were shared by two (one brother and one cousin) of six maternally related males and two (his grandmother and one sister) of seven maternally related females who live in Tunisia. Molecular screening for LHON mutations was carried out from total DNA extracted from blood followed by PCR amplification and restriction endonuclease analysis. The mutations associated with LHON at positions 11778 (*Mae*III+), 3460 (*Bsa*HI−), 3394 (*Hae*III+), 4136 (*Nla*III+), 4216 (*Nla*III+), 4917(*Bfa*I+), 5244 (*Msp*I−), 7444 (*Xba*I−), 9438 (*Hae*III−), 13708 (*Bst*O−), 15257 (*Acc*I−), and 15812 (*Rsa*I−) were excluded. He was homoplasmic for the 14484 (*Bst*OI+) mutation. The presence of the 14484 mutation was confirmed by direct sequencing.

We determined the mtDNA haplotype (Ballinger et al. 1992; Torroni et al. 1993*a,* 1993*b,* 1994*a,* 1994*c,* 1994*d*) on which the 14484 mutation had occurred. Relative to the reference sequence (Anderson et al. 1981), the Tunisian mtDNA harbored an *Mbo*I site gain at np 1899, a *Hpa*I site gain at np 3592, *Hae*III site gains at nps 13803 and 16517, a *Bam*HI site loss at np 14258, a *Dde*I site gain at np 10394, and a combined *Hin*fI site gain at np 16389 and *Ava*II site loss at np 16390. This set of variations is characteristic of the African-specific haplogroup L and reveals that the Tunisian mtDNA is a direct derivative of haplotype 35 previously described in the Senegalese population (Chen et al. 1995) (fig. 1).

This finding has two implications. First, it indicates that the 14484 mutation observed in the Tunisian LHON pedigree is due to an independent mutational event. Second, it shows that the 14484 mutation does not require for disease expression the secondary mtDNA mutations with which it is usually associated in Europeans. In this context, the 14484 mutation meets the requirements for a primary LHON mutation.

Why does the 14484 mutation tend to cluster on a single mtDNA haplogroup (haplogroup J) in Europeans? We envision two possible explanations. First, the occurrence of the 14484 mutation on haplogroup J mtDNAs might make LHON expression more likely than on other haplogroups because of the haplogroup J–specific polymorphisms. Thus, the 14484 mutations observed in affected pedigrees with haplogroup J mtDNAs could represent independent mutational events. If this was the case, then most of the newly occurring 14484 mutations that arise in nonhaplogroup J mtDNAs do not cause LHON and remain undetected in the general European population.

The second possibility is that the 14484 mutation arose once on a haplogroup J mtDNA that has expanded in the population. Riordan-Eva and collaborators (1995) reported that there is a strong male bias of LHON expression in Europeans, which is 2.5:1 for the 11778 mutation, 2:1 for the 3460 mutation, and reaches 5.7:1 for the 14484 mutation. Even a strong selective pressure against a phenotype mainly expressed by males would affect only marginally the genetic fitness of the females who transmit mtDNA molecules with the 14484 mutation. Thus, even though the 14484 mutation is sufficient to cause a severe phenotype, selection mechanisms might have a minimal effect on the maintenance of the mutation in the population. In this context, the excess of 14484 mutations in the European-specific haplogroup J would reflect one mutational event on a single ancestral haplogroup J mtDNA that by drift and founder events has expanded by descent within some European populations.

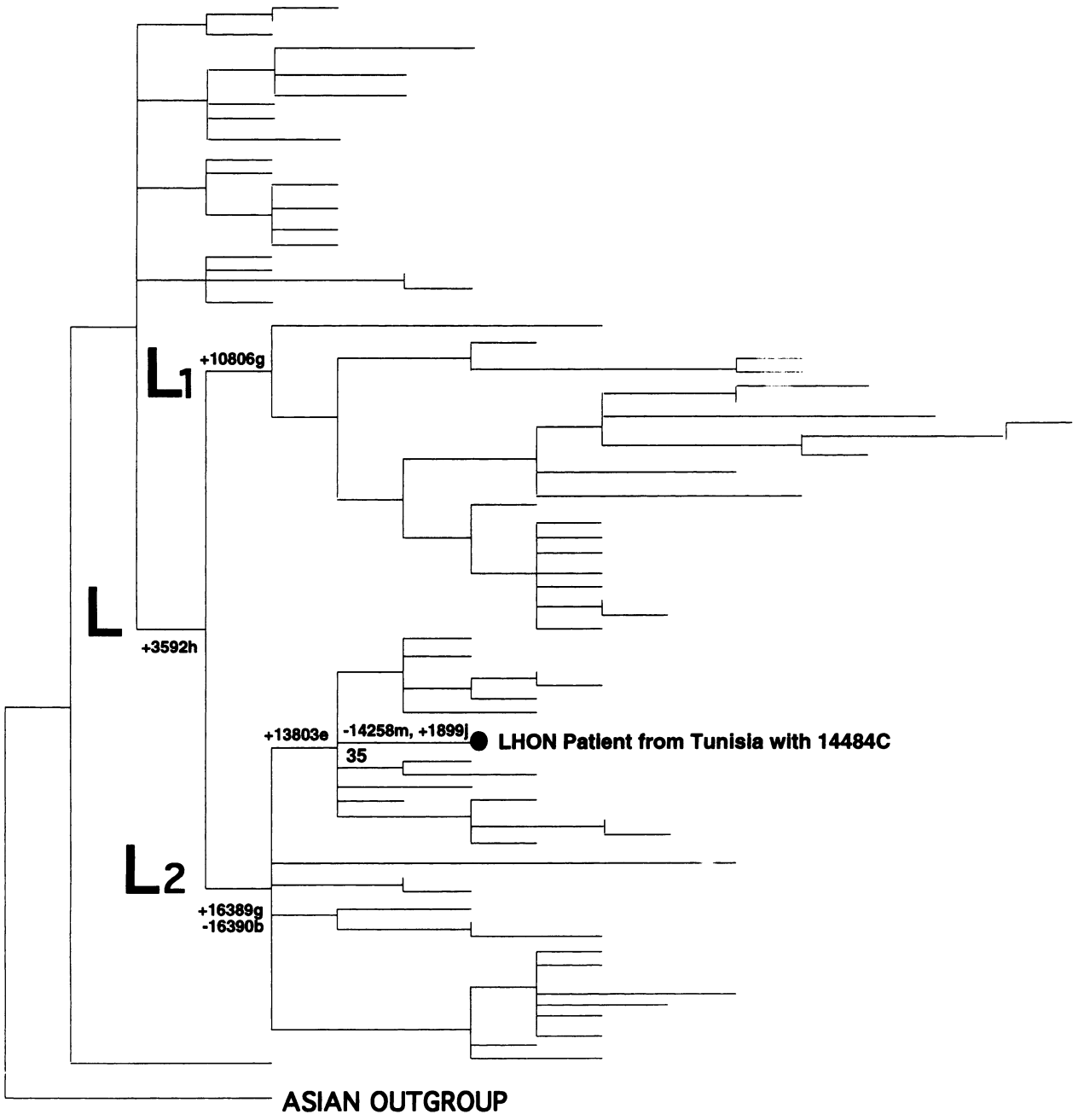


**Figure 1** Maximum parsimony tree, obtained by PAUP analysis (Swofford 1993) and including 79 haplotypes described by Chen et al. (1995) in some African populations and the mtDNA haplotype observed in the LHON patient from Tunisia. The patient's haplotype is a derivative of the haplotype 35 observed in Senegal. The tree is 158 steps in length, has consistency and retention indices of 0.643 and 0.900, respectively, and was rooted as described by Chen et al. (1995). The capital letter L indicates the African-specific haplogroup defined by the 3592 *Hpa*I site. L1 and L2 indicate subhaplogroups within haplogroup L, and the numbers associated with the lowercase letters indicate the restriction sites observed in the LHON patient mtDNA. The restriction enzymes correspond to the following code: b = *Ava*II; e = *Hae*III; g = *Hin*fI; h = *Hpa*I; j = *Mbo*I; and m = *Bam*HI. The horizontal branch lengths are proportional to the number of mutational events that separate the haplotypes.

Data currently available do not permit us to choose between these two different models. However, we believe that additional data on the frequency and distribution of the 14484 mutation in populations of LHON patients from numerous European and non-European countries, the determination of the associated haplotypes, and extensive haplotype analyses of ethnically matched control populations may resolve this issue. In-

deed, if the occurrence of the 14484 mutation on a haplogroup J mtDNA increases the risk of disease expression, the frequency of this mutation among LHON patients from different European populations would depend mainly on the frequency of haplogroup J mtDNAs in each specific population. Preliminary data suggest that haplogroup J frequencies differ in European populations (A. Torroni, K. Huponen, P. Francalacci, M. Petrozzi, L. Morelli, R. Scozzari, D. Obinu, et al., unpublished data), and that this approach could be informative. On the other hand, the same haplotype analysis would also verify whether the accumulation of the 14484 mutation on haplogroup J is due to a founder event. If there was a founder event, it is likely that it occurred at a certain stage of the radiation of European populations. As a consequence, the modern European populations that most directly descend from the ancestral European group in which the founder event occurred should have the highest degree of association between the 14484 mutation and haplogroup J, while this association should be weaker if not absent in other European populations.

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## On the Formation of Nucleosomes within the FMR1 Trinucleotide Repeat

*To the Editor:*

Zhong et al. (1995) presented an intriguing analysis both of the AGG trinucleotides interspersed in the CGG/CCG triplet repeats of the FMR1 gene and of the effect that they may have on trinucleotide-repeat expansion. They suggested that pure FMR1 triplet repeats >50 repeats in length might efficiently form nucleosomes, promoting trinucleotide-repeat expansion through strand slippage or a pause during DNA replication. Several recent papers suggest, however, that the free energy of nucleosome formation on DNA consisting of only guanylate and cytidylate nucleotides is extremely unfavorable (as I shall detail below), because of the inflexibility of the DNA. Expanded CGG/CCG trinucleotide repeats may therefore repress rather than encourage nucleosome formation, and the DNA decondensation and "fragile" chromosome aberration may be a direct consequence of the thermodynamics of DNA bending.

The positioning of nucleosomes on DNA sequences has been investigated by several groups, with the result that preferred sites of nucleosome formation can be predicted with some certainty by consideration of the sequence-dependent free energy ($\Delta G$) of DNA bending (Satchwell and Travers 1989; Sivolob and Khrapunov 1995). This parameter is estimated by the equation $\Delta G = {}^1/_2 kTp\eta^2 L$, where $k$ is Boltzmann's constant, $T$ is the temperature in degrees Kelvin, $p$ is the persistence length of the DNA, $\eta$ is the reciprocal of the radius ($\approx$4.3 nm when packed in a nucleosome), and $L$ is the length of DNA ($\approx$0.34 nm/bp). The terms on the right side of the equation are all straightforward, except for $p$, which is a measure of the stiffness of the DNA and is determined experimentally on synthetic DNA polymers (Hogan and Austin 1987). The energetics of DNA bending within the trinucleotide-repeat regions of the FMR1 and Huntington disease (HD) gene loci can be estimated as shown in figure 1, by plotting the calculated $\Delta G$ for DNA segments, as a function of nucleotide position in the sequence (calculated on a 12-nt window with $T$ = 310°K and by using the persistence-length values $p_{exp}$ for each dinucleotide, as described in table 1 of the report by Sivolob and Khrapunov [1995]). In this graphical representation, values that are higher on the Y-axis (ordinate) result from DNA sequences that are stiffer and hence have a higher (energetically less favorable) $\Delta G$ of bending. On the basis of these calculations, the free energy of DNA bending is ~65 J/nmol/nt in the CGG/CCG repeats of the FMR1 locus (fig. 1, *unbroken line*), compared with 45 J/nmol/nt in the CAG/CTG repeats of the HD locus (fig. 1, *stippled line*). These differences in $\Delta G$ may not seem extraordinary at first glance, since more-extreme values are found intermittently in the 5′ and 3′ flanking sequences of both repeats, but the expansion of the respective trinucleotide repeats over several hundreds or thousands of nucleotides would strongly repress (in the case of FMR1) or encourage (in the case of HD) nucleosome formation. Indeed, in the