proach in which mtDNA analysis will be equivalent to other methodologies. In this regard, and paraphrasing Torroni and Wallace (1995), we would also like to caution the scholars of mtDNA analysis against thinking that this methodology is the panacea that will resolve all our anthropological doubts.

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References

Bailliet G, Rothhammer F, Carnese FR, Bravi CM, Bianchi NO (1994) Founder mitochondrial haplotypes in Amerindian populations. Am J Hum Genet 55:27–33

Bianchi NO, Bailliet G. Powers and pitfalls of the use of mitochondrial DNA in anthropology. In: Barton S, Rothhammer F, Schull W (eds) Patterns of morbidity in Andean aboriginal populations: 8000 years of evolution. (in press)

Cann RL, Stoneking M, Wilson AC (1987) Mitochondrial DNA and human evolution. Nature 325:31–36

Gill P, Ivanov PL, Kimpton C, Piercy R, Benson N, Tully G, Evett I, et al (1994) Identification of the remains of the Romanov family by DNA analysis. Nat Genet 6:130–135

Horai S, Kondo R, Nakagawa-Hattori Y, Hayashi S, Sonoda S, Tajima K (1993) Peopling of the Americas, founded by four major lineages of mitochondrial DNA. Mol Biol Evol 10:23–47

Koheler CM, Lindberg GL, Brown DR, Beitz DC, Freeman AE, Mayfield JE, Myers AM (1991) Replacement of bovine mitochondrial DNA by a sequence variant within one generation. Genetics 129:247–255

Merriwether DA, Rothhammer F, Ferrell RE (1993) Mitochondrial DNA D-loop sequence variation in native South Americans. Am J Hum Genet Suppl 53:833

Schurr TG, Ballinger SW, Gan Y-Y, Hodge JA, Merriwether DA, Lawrence DN, Knowler WC, et al (1990) Amerindian mitochondrial DNAs have rare Asian mutations at high frequencies, suggesting they derived from four primary maternal lineages. Am J Hum Genet 46:613–623

Stewart CB (1993) The powers and pitfalls of parsimony. Nature 361:603–607

Stone AC, Stoneking M (1993) Ancient DNA from a pre-Columbian Amerind population. Am J Phys Anthropol 92:463–471

Stoneking M, Sherry ST, Redd AJ, Vigilant L (1992) New approaches to dating suggest a recent age for the human mtDNA ancestor. Phil Trans R Soc Lond B 337:167–175

Torroni A, Neel JV, Barrantes R, Schurr TG, Wallace DC (1994) Mitochondrial DNA "clock" for the Amerinds and its implications for timing their entry into North America. Proc Natl Acad Sci USA 91:1158–1162

Torroni A, Schurr TG, Cabell MF, Brown MD, Neel JV, Larsen M, Smith DG, et al (1993*a*) Asian affinities and continental radiation of the four founding Native American mtDNAs. Am J Hum Genet 53:563–590

Torroni A, Schurr TG, Yang CC, Szathmary EJE, Williams RC, Schanfield MS, Troup GA, et al (1992) Native American mitochondrial DNA analysis indicates that the Amerind and the Nadene populations were founded by two independent migrations. Genetics 130:153–162

Torroni A, Sukernik RI, Schurr TG, Starikovskaya YB, Cabell MF, Crawford MH, Comuzzie AG, et al (1993*b*) mtDNA variation of aboriginal Siberians reveals distinct genetic affinities with native Americans. Am J Hum Genet 53:591–608

Torroni A, Wallace DC (1995) mtDNA haplogroups in Native Americans. Am J Hum Genet 56:1234–1236 (in this issue)

Vigilant L, Pennington R, Hardpening H, Kocher TD, Wilson AC (1989) Mitochondrial DNA sequences in single hairs from a southern African population. Proc Natl Acad Sci USA 86:9350–9354

Vigilant L, Stoneking M, Harpending H, Hawkes K, Wilson AC (1991) African populations and the evolution of human mitochondrial DNA. Science 253:1503–1507

0002-9297/95/5605-0029$2.00

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A Mitochondrial Mutation at nt 9101 in the ATP Synthase 6 Gene Associated with Deficient Oxidative Phosphorylation in a Family with Leber Hereditary Optic Neuroretinopathy

To the Editor:

Leber hereditary optic neuroretinopathy (LHON) is a maternally inherited ocular disease resulting in bilateral optic atrophy in young adults. Several mtDNA point mutations have been proposed as being causative for LHON, all in complex I, III, or IV of the respiratory chain. The ND4/11778 mutation accounts for ~50% of all LHON families (Wallace et al. 1988; Vilkki et al. 1990), the ND1/3460 mutation (Howell et al. 1991; Huoponen et al. 1991) is detected in ~15 % of cases, and ~10% of LHON families have the ND6/14484 mutation (Mackey and Howell 1992). All these mutations are restricted to LHON families, and they change evolutionarily conserved amino acids. Furthermore, these primary mutations have never been observed to occur simultaneously.

Besides the primary mutations, several other replacement mutations have been found in LHON families (Brown et al. 1992; Huoponen et al. 1993). These muta-

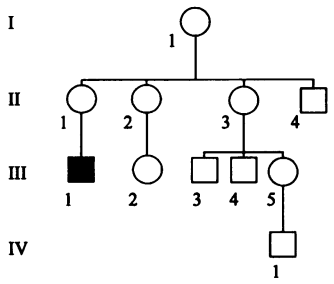

Figure 1 Pedigree of the LHON family with ATPase6/9101 mutation. The blackened symbol indicates the proband with optic atrophy.

tions are also detected at low frequency in control individuals, and they change evolutionarily less conserved amino acids.

Thirteen of the 24 Finnish LHON families harbor the ND4/11778 mutation, and 3 have the ND1/3460 mutation. Sequencing of the protein-coding mitochondrial genes for complex I (genes ND1–ND6 and ND4L) did not reveal any new candidate for primary mutation in the remaining eight families (Huoponen et al. 1993). The search for pathogenic mutations was thus extended to the genes for cytochrome b ATPase subunits 6 and 8 and for cytochrome c oxidase subunits I–III. Here we report a new mutation that is possibly associated with LHON: a T-to-C transition at nt 9101 at residue 192, resulting in the replacement of an isoleucine by a threonine in the ATPase 6 subunit gene.

The family with the ATPase 6/9101 mutation represents possible LHON according to the classification of Vilkki et al. (1989). Patient III-1 (in family 6 in Huoponen et al. 1993) (fig. 1) is the only affected individual in the family. He was affected at the age of 21 years, and his disease had a typical acute stage with peripapillary microangiopathy. Eye examination of his 79-year-old mother was unrevealing, and she had no peripapillary microangiopathy.

The base substitution at nt 9101 abolished a restriction site for *Mbo*II and created a new site for *Hph*I, providing an easy method for detection of the mutation. *Mbo*II digestion of the PCR-amplified 320-bp fragment from normal DNA produced fragments of 120 and 200 bp, whereas a 320-bp fragment was obtained from mutated DNA (fig. 2*a*). In contrast, *Hph*I digestion produced an intact fragment from normal DNA and produced fragments of 120 and 200 bp from mutated DNA (fig. 2*b*). Four additional maternal members of the family, the probands of the other 23 Finnish LHON families, and 100 unrelated control individuals of Finnish origin were screened for the presence of the mutation. The mutation was detected in all maternal members of family 6 but not in the other individuals tested, and it has not been reported in any of the published surveys of mtDNA nucleotide variations.

The sequencing revealed four other replacement mutations in the same individual, at the following positions: nt 8860 (Thr→Ala) in the gene encoding ATPase subunit 6; nt 14766 (Ile→Thr) and nt 15326 (Thr→Ala), both in the cytochrome b gene; and nt 9559 (Arg→Pro) in the cytochrome c oxidase subunit III gene. All these base changes are reported to be normal variants without pathological significance (Marzuki et al. 1991). Other maternal family members were not screened for these mutations. No replacement mutations were previously detected in any of the seven genes for complex I subunits (Huoponen et al. 1993).

By means of Southern blotting the proband and three unaffected maternal family members were shown to be homoplasmic for the ATPase 6/9101 mutation. This implies that the mutation has not arisen recently.

The nt 9101 base change is the first LHON-associated ATPase 6 subunit mutation. The ATP-synthesizing complex (F_1F_0-ATPase) of mitochondria couples the electrochemical proton gradient, generated across the inner mitochondrial membrane by the respiratory chain, to synthesis of ATP. It is composed of a membranous F_0 segment, which encompasses the proton channel, and of the F_1 portion, which catalyzes ATP synthesis from ADP and P_i on the matrix side of the inner membrane. The F_0 segment is formed by three polypeptides; subunits 6 and 8 are encoded by mtDNA, and subunit 9 is encoded by nuclear DNA (Fillingame et al. 1992). Residue 192 in subunit 6 is weakly conserved; in man and in sea urchin it is isoleucine, but in most other species

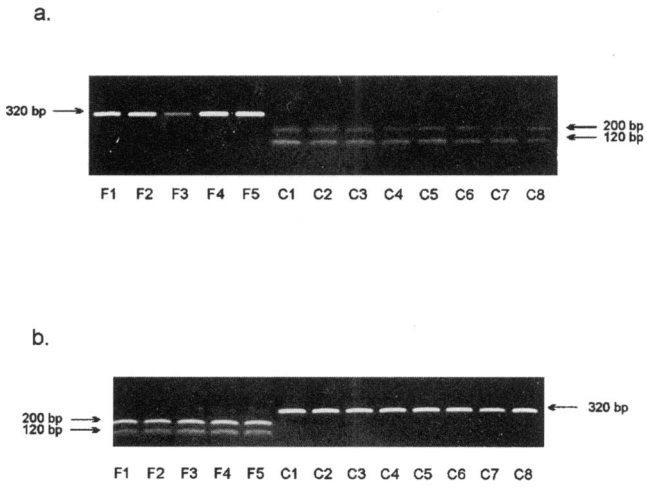

Figure 2 *a*, mtDNA mutation at position 9101, detected by *Mbo*II analysis. In normal mtDNA, *Mbo*II cuts the PCR-amplified 320-bp fragment into fragments of 120 and 200 bp. The mutation eliminates the *Mbo*II restriction site and results in an intact 320-bp fragment. Samples F1–F5 are from family 6, and samples C1–C8 are control samples. *b*, Same samples as in panel *a*, digested with *Hph*I. In normal mtDNA, *Hph*I restriction-enzyme digestion results in an intact 320-bp fragment. The mutation creates a new restriction site for *Hph*I. Samples with the 9101 mutation are cut into fragments of 120 and 200 bp.

(bovine, mouse, rat, chicken, and *Xenopus laevis*) it is threonine, as in the LHON case studied here.

Oxidative phosphorylation was studied in mitochondria from cultured lymphoblasts of patient III-1, an unaffected family member II-1, and two controls, by measuring the rates of ATP synthesis and electron transfer through complexes I and III simultaneously. In mitochondria carrying the ATPase 6/9101 mutation, the efficiency of oxidative phosphorylation was reduced by 40%–50%, as revealed by a lowered ATP/2e$^-$ ratio (III-1, 0.75 ± 0.07 [n = 3], and II-1, 0.78 ± 0.06 [n = 3]; controls, 1.38 ± 0.14 [n = 7]), which relates the rate of ATP synthesis to the rate of electron transfer. The oligomycin-sensitive ATPase activity due to the F_0F_1 complex was normalized to succinate dehydrogenase activity of the preparation, and a 20% decline was observed in the mutant preparation relative to that of the controls (III-1, 0.282 ± 0.025 [n = 3]; controls, 0.336 ± 0.055 [n = 3]).

Two other mutations in ATPase subunit 6 have previously been associated with human disease. Heteroplasmic substitutions of Leu for Arg or Pro (de Vries et al. 1993) at residue 156 have been demonstrated in patients with either NARP (neurogenic muscle weakness, ataxia, and retinitis pigmentosa) or Leigh disease. We report now a new candidate for a primary mutation in LHON in a patient with typical symptoms of the disease. Although the site of the mutation is not in the conserved region of the ATPase subunit 6 gene, the biochemical defect in oxidative phosphorylation is specifically traced to complex V. To confirm the etiological role of the ATPase 6/9101 mutation in LHON, screening of the mutation is needed in LHON families from different populations.

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References

Brown MD, Voljavec AS, Lott MT, Torroni A, Yang C-C, Wallace DC (1992) Mitochondrial DNA complex I and III mutations associated with Leber's hereditary optic neuropathy. Genetics 130:163–173

de Vries DD, van Engelen BGM, Gabreels FJM, Ruitenbeek W, van Oost BA (1993) A second missense mutation in the mitochondrial ATPase 6 gene in Leigh's syndrome. Ann Neurol 34:410–412

Fillingame RH, Girvin ME, Fraga D, Zhang Y (1992) Correlations of structure and function in H^+ translocating subunit c of F_1F_0 ATP synthase. Ann N Y Acad Sci 671:323–333

Howell N, Bindoff LA, McCullough DA, Kubacka I, Poulton J, Mackey D, Taylor L, et al (1991) Leber hereditary optic neuropathy: identification of the same mitochondrial ND1 mutation in six pedigrees. Am J Hum Genet 49:939–950

Huoponen K, Lamminen T, Juvonen V, Aula P, Nikoskelainen EK, Savontaus M-L (1993) The spectrum of mitochondrial DNA mutations in families with Leber hereditary optic neuroretinopathy. Hum Genet 92:379–384

Huoponen K, Vilkki J, Aula P, Nikoskelainen EK, Savontaus M-L (1991) A new mtDNA mutation associated with Leber hereditary optic neuroretinopathy. Am J Hum Genet 48:1147–1153

Mackey D, Howell N (1992) A variant of Leber hereditary optic neuropathy characterized by recovery of vision and by an unusual mitochondrial genetic etiology. Am J Hum Genet 51:1218–1228

Marzuki S, Noer AS, Lertrit P, Thyagarajan D, Kapsa R, Utthanapol P, Byrne E (1991) Normal variants of human mitochondrial DNA and translation products: the building of a reference data base. Hum Genet 88:139–145

Vilkki J, Savontaus M-L, Nikoskelainen EK (1989) Genetic heterogeneity in Leber hereditary optic neuroretinopathy revealed by mitochondrial DNA polymorphism. Am J Hum Genet 45:206–211

——— (1990) Segregation of mitochondrial genomes in a heteroplasmic lineage with Leber hereditary optic neuroretinopathy. Am J Hum Genet 47:95–100

Wallace DC, Singh G, Lott MT, Hodge JA, Schurr TG, Lessa AMS, Elsas LJ II, et al (1988) Mitochondrial DNA mutation associated with Leber's hereditary optic neuroretinopathy. Science 242:1427–1430

0002-9297/95/5605-0030$2.00

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Exclusion of Chromosome 1q21-q31 from Linkage to Three Pedigrees Affected by the Pigment-Dispersion Syndrome

To the Editor:

The pigment-dispersion syndrome is a form of open-angle glaucoma that usually affects individuals in the first 3 decades of life. In addition to the typical optic-nerve degeneration seen in all types of glaucoma, the pigment-dispersion syndrome is characterized by distinctive clinical features including the deposition of pig-