Herbots H, Demaine A, Papiha S, et al (1996) Paleolithic and Neolithic lineages in the European mitochondrial gene pool. Am J Hum Genet 59:185–203

Address for correspondence and reprints: Dr. Martin Richards, Department of Cellular Science, Institute of Molecular Medicine, John Radcliffe Hospital, Headington, Oxford OX3 9DS, United Kingdom. E-mail: mrichard@worf.molbiol.ox.ac.uk

―――――――

Reply to Hofmann et al.

To the Editor:

In the June issue of the *Journal*, Hofmann et al. (1997) commented on our study of Leber hereditary optic neuropathy (LHON; MIM 535000 [http://www3.ncbi .nlm.nih.gov:80/htbin-post/Omim/dispmim?535000]) multigeneration pedigrees (Mackey et al. 1996). Hofmann et al. made two main points in their letter, the first of which was that a mutation at nucleotide 15257 of the mitochondrial cytochrome *b* gene has a pathogenic role in LHON. This is a long-standing and unresolved controversy that epitomizes the pitfalls that beset the identification of pathogenic mtDNA mutations. Their second point, which is not an issue that we addressed, was that "so-called" secondary LHON mutations, in their terminology, play an etiologic role in other neurological disorders.

There is broad agreement that mtDNA mutations at nucleotides 3460, 11778, and 14484 are pathogenic LHON mutations, but there is disagreement over the pathogenic role of the 15257 mutation (Howell 1994, 1997*a*; Oostra et al. 1994). In our previous reports, including that by Mackey et al. (1996), the term "primary" was applied to the 3460, 11778, and 14484 LHON mutations. LHON is maternally inherited, but the penetrance is incomplete. The pathogenic mtDNA mutation is thus the predominant risk factor, but additional etiologic factors are required for manifestation of the optic neuropathy (Howell et al. 1997*a*, 1997*b*). In that sense, the 3460, 11778, and 14484 mtDNA mutations have a *primary* pathogenic role in LHON. Several additional mtDNA mutations have been identified that may have an etiologic or pathogenic role in LHON, including some that may augment or modify the phenotypic effects of the three known pathogenic mutations (see below). As a result of this uncertainty, the nomenclature has become commensurately more complicated. For example, Brown and Wallace (1994) list 16 mutations that have primary, secondary, or intermediate roles in LHON, although

even this list is now incomplete (e.g., see Howell et al. 1998, and references therein).

The purpose of our previous study (Mackey et al. 1996) was to identify the pathogenic mtDNA mutations in LHON pedigrees. A total of 159 families (comprising ~12,000 maternal relatives) from Australia and northern Europe were analyzed, because these are countries where extensive genealogies are more easily obtained. We limited our study to large multigeneration LHON families, to avoid the ambiguities that arise with singleton cases of bilateral optic neuropathy in which maternal inheritance is lacking. The majority of sporadic cases of a LHON-like optic atrophy are not associated with the 3460, 11778, or 14484 LHON mutations (Chan et al. 1996). *None* of these 159 LHON families carried the 15257 mutation in the absence of one of the three previously established LHON mutations, although it was associated with one of the three mutations in six LHON families. This association has also been found in other LHON families (Howell et al. 1993; Oostra et al. 1994), as well as among those analyzed by Hofmann et al. (1997). The penetrance of pathogenic mutations is not increased in the LHON families whose mtDNAs also harbor the 15257 mutation (e.g., see Howell et al. 1993; Torroni et al. 1997). These negative results argue against a pathogenic role for the 15257 mutation, because LHON is a disorder whose penetrance is particularly dependent on the action of secondary etiologic factors (Howell 1997*a*, 1997*b*).

In contrast to our results, Obermaier-Kusser et al. (1994) reported a LHON family, with multiple affected family members that span multiple generations, that carries the 15257 mutation but not one of the three previously identified pathogenic LHON mutations. Hofmann et al. (1997) report a total of 55 optic neuropathy index cases, 3 of which are 15257 plus 11778 and 6 of which are 15257 plus 14484, but 5 of which are "15257 only." It is not clear whether these 15257-only cases are distant relatives of the LHON family described elsewhere (Obermaier-Kusser et al. 1994) or have affected maternal relatives. It is precisely because of the experimental and analytical difficulties inherent to singleton cases that we undertook our study of multigeneration families. More important, sequencing analysis has not been performed, either for the 15257-only LHON family or for the new 15257 cases, and the presence of a rare, unidentified pathogenic mutation cannot be ruled out.

The 15257 mutation has been detected at a low frequency (Brown et al. 1992; Kalman et al. 1995; and especially see Torroni et al. 1997) in normal control subjects, a result that argues against a pathogenic role. However, population surveys of normal controls should capture individuals who harbor a pathogenic LHON mutation but who, because of the incomplete penetrance, are not clinically affected. The 3460, 11778, and

14484 LHON mutations, which tend to produce a relatively high-penetrance form of LHON (Mackey 1994; Chan et al. 1996), have *not* yet been detected in such surveys. If the 15257 mutation produces an unusually low-penetrance form of LHON, this would explain both the absence of 15257-only multigeneration LHON families in our survey and the presence of the 15257 mutation in normal control populations. That explanation, however, does not fit the observation that the 15257 LHON family of Obermaier-Kusser et al. (1994) has a *high* penetrance.

Hofmann et al. (1997) also discuss haplogroup clustering and the 15257 mutation. Haplogroup clustering was first reported by Johns and Berman (1991), who showed that the frequency of what they termed *secondary* LHON mutations at nucleotides 4216 and 13708 were associated more often with 11778 LHON patients than with normal controls. It is now recognized that the 4216 and 13708 sequence changes define one of the major European haplogroups (designated "A" by Hofmann et al. [1996], "2A" by Richards et al. [1996], and "J" by Torroni et al. [1996]). Both the 11778 and 14484 mutations cluster, or are associated preferentially, with this haplogroup, and ~75% of 14484 LHON patients or pedigrees carry mitochondrial genomes that belong to this haplogroup (Johns 1994; Brown et al. 1997; Torroni et al. 1997). There is no satisfactory explanation for this clustering, although it has been proposed that one or more of the mtDNA sequence changes in this haplogroup markedly increases the penetrance of the 11778 and 14484 LHON mutations, particularly in the case of the latter (Brown et al. 1997; Torroni et al. 1997). The 15257 mutation, with rare exception, is also associated with this haplogroup (Howell et al. 1995; Brown et al. 1997; Torroni et al. 1997).

In addition to haplogroup J, there is a second haplogroup, which carries the mutation at nucleotide 4216 and a mutation at nucleotide 4917 (designated "B" by Hofmann et al. [1997], "2B" by Richards et al. [1996], and "T" by Torroni et al. [1996]). We (Howell et al. 1995), Hofmann et al. (1997), and Richards et al. (1996) agree that European haplogroups J and T are members of the same phylogenetic cluster (but, for the alternative view that the two haplogroups are less closely related, see Torroni et al. [1996, 1997]). It was initially concluded that there was also clustering of LHON mutations with the 4216+4917 combination of mutations (Johns and Berman 1991), but more-recent results do not support this conclusion (Torroni et al. 1997). Hofmann et al. (1997) report two LHON patients in whom the 15257 LHON mutation is associated with the mutation at nucleotide 4917. Furthermore, they state that *one* of these patients did not carry a LHON mutation at nucleotide 3460, 11778, or 14484. It is difficult to assess the ramifications of those results, but Torroni et

al. (1997) have reported the 4917+15257 combination in a *normal* control. Furthermore, Torroni et al. (1997) have shown that the mitochondrial genomes from their LHON pedigrees cluster with the haplogroup J subbranch that does *not* carry the 15257 mutation (see their fig. 2), results that imply that the 15257 mutation does not increase LHON penetrance (see above).

The second major point raised by Hofmann et al. (1997, p. 1540) is that secondary LHON mutations are frequently associated with non-LHON neurological disorders. As evidence for this proposition, they cite recent studies that found an increased frequency of the 15257 and 13708 mutations, both in a cohort of patients with multiple sclerosis (MS) and visual impairment (Mayr-Wohlfart et al. 1996) and in a group of patients with varied neurodegenerative disorders (Rödel et al. 1996). However, even if the results of Kalman et al. (1995) and Mayr-Wohlfart et al. (1996) are pooled, there is no statistically significant association between the 15257 mutation and MS (data not shown). Second, there is no significant association between the 13708 mutation and MS in the pooled data from these two studies, nor when they are pooled with the much larger study by Kellar-Wood et al. (1994). It is also possible to use the pooled data of Kalman et al. (1995) and Mayr-Wohlfart et al. (1996) to show that there is no significant association between MS and haplotype J (data not shown). Rödel et al. (1996) have reported that 7 of 200 neurology patients from a southern German population harbor mtDNAs with the 13708+15257 array of secondary LHON mutations, although the frequency of this combination of sequence changes among normals from the same geographic region is not specified. Richards et al. (1996) have shown that haplogroup J (which they term "2A") has a heterogeneous geographic distribution throughout Europe and the Middle East, and the results of Rödel et al. (1996) thus require wider surveys of haplogroup J population samples.

Hofmann et al. (1997) have reported that the mtDNA from five of their eight patients with DIDMOAD (diabetes insipidus, diabetes mellitus, optic atrophy, and deafness) belong to the 4216+4917 haplogroup (haplogroup T of Torroni et al. [1996]), a frequency (63%) much higher than the frequency (9%) of this haplogroup among the controls in their study. In addition, there appears to be an increased frequency of MS patients with mtDNAs who belong to this haplogroup (Kalman et al. 1995; Mayr-Wohlfart et al. 1996). In these two studies, the pooled frequencies were 16/153 in MS patients and 7/174 in the controls ($P < .05$, as determined by a χ^2 test). Hofmann et al. thus conclude that the 4216, 4917, and 13708 sequences are not secondary LHON mutations but that, instead, they are mtDNA mutations that have an etiologic role (possibly secondary) in a variety of neurological disorders. We believe that such a con-

clusion is premature and that more-extensive screening of both patients and controls from geographically diverse European subpopulations is required. For example, mtDNA haplotype analysis of another set of 28 Caucasian DIDMOAD patients has not revealed haplotype clustering (J. Poulton and T. Barratt, unpublished data). Especially within small geographic areas, there may be, between *nuclear* genotypes and mitochondrial haplotypes, preferential associations that may be statistically significant but that do not signal an etiologic role for the mitochondrial genome in the disease process (for an analysis of the relative nuclear and mitochondrial genetic contributions from founders in isolated populations, see Heyer [1995]).

In summary, it is our view that the available data do not *yet* indicate that the 4216, 4917, 13708, and 15257 mutations (and any sequence changes associated with haplogroups J and T) are pathogenic, either in LHON or in other neurological abnormalities. We agree with Hofmann et al. (1997) that further investigation is warranted.

Dᴀᴠɪᴅ Mᴀᴄᴋᴇʏ,[1] Rᴏᴇʟᴏғ-Jᴀɴ Oᴏsᴛʀᴀ,[3]
Tʜᴏᴍᴀs Rᴏsᴇɴʙᴇʀɢ,[6] Eᴇᴠᴀ Nɪᴋᴏsᴋᴇʟᴀɪɴᴇɴ,[7]
Jᴏᴀɴɴᴀ Pᴏᴜʟᴛᴏɴ,[9] Tɪᴍ Bᴀʀʀᴀᴛᴛ,[9] Pɪᴇᴛᴇʀ Bᴏʟʜᴜɪs,[4]
Søʀᴇɴ Nøʀʙʏ,[10] Mᴀʀᴊᴀ-Lɪɪsᴀ Sᴀᴠᴏɴᴛᴀᴜs,[8]
Cʜʀɪsᴛᴏᴘʜᴇʀ Cʜᴀɴ,[2] ᴀɴᴅ Nᴇɪʟ Hᴏᴡᴇʟʟ[11]

[1]*Departments of Ophthalmology and Paediatrics, The University of Melbourne, and Menzies Centre for Population Health Research, The University of Tasmania, Hobart, and [2]Department of Ophthalmology, The University of Melbourne, Melbourne; [3]Department of Anatomy and Embryology, University of Amsterdam, and [4]Academic Medical Centre, Laboratory Neurointuigen, Amsterdam; [6]National Eye Clinic for the Visually Impaired, Hellerup, Denmark; [7]Department of Ophthalmology, Turku University, and [8]Department of Medical Genetics, Turku University Central Hospital, Turku, Finland; [9]Department of Paediatrics, The John Radcliffe Hospital, Oxford; [10]Department of Forensic Pathology, The University of Copenhagen, Copenhagen; and [11]Department of Radiation Oncology and Department of Human Biological Chemistry and Genetics, The University of Texas Medical Branch, Galveston*

References

Brown MD, Sun F, Wallace DC (1997) Clustering of Caucasian Leber hereditary optic neuropathy patients containing the 11778 or 14484 mutations on an mtDNA lineage. Am J Hum Genet 60:381–387

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

Brown MD, Wallace DC (1994) Spectrum of mitochondrial DNA mutations in Leber's hereditary optic neuropathy. Clin Neurosci 2:138–145

Chan C, Mackey DA, Byrne E (1996) Sporadic Leber hereditary optic neuropathy in Australia and New Zealand. Aust NZ J Ophthalmol 24:7–14

Heyer E (1995) Mitochondrial and nuclear genetic contribution of female founders to a contemporary population in northeast Quebec. Am J Hum Genet 56:1450–1455

Hofmann S, Bezold R, Jaksch M, Kaufhold P, Obermaier-Kusser B, Gerbitz K-D (1997) Disease relevance of the so-called secondary Leber hereditary optic neuropathy mutations. Am J Hum Genet 60:1539–1542

Howell N (1994) Mitochondrial gene mutations and human diseases: a prolegomenon. Am J Hum Genet 55:219–224

——— (1997a) Leber hereditary optic neuropathy: how do mitochondrial DNA mutations cause degeneration of the optic nerve? J Bioenerg Biomembr 29:165–173

——— (1997b) Leber hereditary optic neuropathy: mitochondrial mutations and degeneration of the optic nerve. Vision Res 37:3495–3507

Howell N, Bogolin C, Jamieson R, Marenda DR, Mackey DA (1998) mtDNA mutations that cause optic neuropathy: how do we know? Am J Hum Genet 62:196–202

Howell N, Kubacka I, Halvorson S, Howell B, McCullough DA, Mackey K (1995) Phylogenetic analysis of mitochondrial genomes from Leber hereditary optic neuropathy pedigrees. Genetics 140:285–302

Howell N, Kubacka I, Halvorson S, Mackey D (1993) Leber's hereditary optic neuropathy: the etiological role of a mutation in the mitochondrial cytochrome b gene. Genetics 133:133–136

Johns DR (1994) Genotype-specific phenotypes in Leber's hereditary optic neuropathy. Clin Neurosci 2:146–150

Johns DR, Berman J (1991) Alternative simultaneous complex I mitochondrial DNA mutations in Leber's hereditary optic neuropathy. Biochem Biophys Res Comm 174:1324–1330

Kalman B, Lublin FD, Alder H (1995) Mitochondrial DNA mutations in multiple sclerosis. Multiple Sclerosis 1:32–36

Kellar-Wood H, Robertson N, Govan GG, Compston AS, Harding AE (1994) Leber's hereditary optic neuropathy mitochondrial DNA mutations in multiple sclerosis. Ann Neurol 36:109–112

Mackey DA (1994) Epidemiology of Leber's hereditary optic neuropathy in Australia. Clin Neurosci 2:162–164

Mackey DA, Oostra R-J, Rosenberg T, Nikoskelainen E, Bronte-Stewart J, Poulton J, Harding AE, et al (1996) Primary pathogenic mtDNA mutations in multigeneration pedigrees with Leber hereditary optic neuropathy. Am J Hum Genet 59:481–485

Mayr-Wohlfart U, Paulus C, Menneberg A, Rödel G (1996) Mitochondrial DNA mutations in multiple sclerosis patients with severe optic involvement. Acta Neurol Scand 94: 167–171

Obermaier-Kusser B, Lorenz B, Schubring S, Paprotta A, Zerres K, Meitinger T, Meire F, et al (1994) Features of mtDNA mutation patterns in European pedigrees and sporadic cases

with Leber hereditary optic neuropathy. Am J Hum Genet 55:1063–1066

Oostra R-J, Bolhuis PA, Zorn-Ende I, de Kok-Nazaruk MM, Bleeker-Wagemakers EM (1994) Leber's hereditary optic neuropathy: no significant evidence for primary or secondary pathogenicity of the 15257 mutation. Hum Genet 94: 265–270

Richards M, Côrte-Real H, Forster P, Macauley V, Wilkinson-Herbots H, Demaine A, Papiha S, et al (1996) Paleolithic and Neolithic lineages in the European mitochondrial gene pool. Am J Hum Genet 59:185–203

Rödel G, Laubhan R, Scheuerle A, Skowronek P, Haferkamp O (1996) Association of the LHON 13708 and 15257 mitochondrial DNA mutations with neurodegenerative diseases distinct from LHON. Eur J Med Res 1:491–494

Torroni A, Huoponen K, Francalacci P, Petrozzi M, Morelli L, Scozzari R, Obinu D, et al (1996) Classification of Europeans mtDNAs from an analysis of three European populations. Genetics 144:1835–1850

Torroni A, Petrozzi M, D'Urbano L, Sellitto D, Zeviani M, Carrara F, Carducci C, et al (1997) 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 60: 1107–1121

Address for correspondence and reprints: Dr. Neil Howell, Biology Division 0656, Department of Radiation Oncology, The University of Texas Medical Branch, Galveston, TX 77555-0656. E-mail: nhowell@utmb.edu

---

Characterization of 10p Deletions Suggests Two Nonoverlapping Regions Contribute to the DiGeorge Syndrome Phenotype

To the Editor:

DiGeorge syndrome (DGS; MIM 188400 [http://www3.ncbi.nlm.nih.gov:80/htbin-post/Omim/dispmim?188400]) is a developmental-field defect characterized by abnormalities of structures derived from the pharyngeal arches and pouches (for a review, see Driscoll and Emanuel 1996). The vast majority of individuals with DGS have been found to have deletions of chromosomal region 22q11.2. However, a small number of patients have been shown to have deletions of chromosome 10p, with normal chromosome 22s (for a review, see Greenberg et al. 1988). We report here the location and extent of the deletion on chromosome 10, determined by means of a combination of heterozygosity tests and FISH analysis, in five DGS patients. Our results do not support the existence of a single, commonly deleted region on

10p in these five patients. Rather, they suggest that deletion of more than one region on chromosome 10p can be associated with the DGS phenotype.

We examined the extent of the chromosome-10p deletions in five patients. Phenotypic characterizations of three (GM6936, CH92-304, and CH95-199) of the five patients were reported elsewhere, by Greenberg et al. (1986), by Monaco et al. (1991) and Pignata et al. (1996), and by Lipson et al. (1996), respectively. Molecular characterizations of the deletions present in GM6936 and CH95-199 were also reported by Daw et al. (1996). Patient CH95-199 is designated as "P3" in Daw et al. (1996). The remaining two patients were referred to us after their deletions were detected by means of high-resolution cytogenetic analysis. All of the patients exhibit at least one of the classic features of DGS (cardiac defect, hypocalcemia, and/or immune defect). Clinical findings are summarized in table 1.

We first performed heterozygosity mapping by use of markers between loci D10S249 and D10S213. These sequence-tagged sites (STSs) correspond roughly to the cytogenetic location 10p15-10p12 (Chumakov et al. 1995). Three of the five patients had a single allele for the three most distal markers, D10S249, D10S591, and D10S189; this finding is consistent with a terminal deletion of 10p (table 2). The remaining two patients were heterozygous, at loci D10S249, D10S591, and D10S189, but had single alleles for a series of more centromeric markers (table 2); this finding is consistent with the presence of an interstitial deletion. As shown in table 2, there is no marker for which all patients have a single allele, which suggests that there is no common region-of-deletion overlap among these five patients.

To confirm the heterozygosity-mapping results, we performed FISH analysis on these five patients, using YACs from the region. All YACs were from the CEPH/Genethon megaYAC library, except 194G1, which was isolated from the smaller-insert CEPH library. FISH analysis confirmed and extended the results obtained from the heterozygosity tests: there is no common region of overlap among all five patients (fig. 1). Four of the five patients have a common region of deletion that includes the shortest region-of-deletion overlap (SRO) described in Daw et al. (1996). Two of these four patients, GM6936 and CH95-199 ("P3"), were included in Daw et al. (1996), and the other two do not narrow the SRO further. Patient CH92-092, although without a deletion for this SRO, does share an extensive region of deletion with the other two patients with terminal deletions. Further, it is possible that there is a small region-of-deletion overlap between CH92-092 and CH92-304. The endpoints of the deletions in these two patients could not be defined precisely because the YAC contig is not continuous from D10S1431 to D10S226. On the basis of the results presented here, it is not possible to attribute