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Gene defects in Leber hereditary optic neuroretinopathy

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Introduction

Leber hereditary optic neuroretinopathy (LHON) is the most prevalent human disease caused by a mutation in mitochondrial genes. Clinical presentation of LHON includes acute or subacute, usually permanent visual loss caused by severe bilateral optic atrophy and large centrocecal scotomata. The disease primarily affects young, otherwise healthy males at their adolescence; only approx. 15% of the patients are women [1]. LHON is maternally inherited, men never transmit the disease to their children. The concept of mitochondrial transmission of LHON is strongly supported by recent molecular genetic findings.

Gene defects in LHON

The mitochondrial origin of LHON was demonstrated by the discovery of a base change in the mitochondrial DNA at nt11778 [2]. The mutation has never been found in control individuals from various ethnic groups [3,4]. The mutation changes a G to an A converting an evolutionarily highly conserved arginine to a histidine in ND4, one of the 7 subunits of the mitochondrial NADH-ubiquinone oxidoreductase that are encoded in mtDNA. The mutation abolishes a *Sfa*NI restriction site and creates an *Mae*III site, thereby enabling specific assays for its detection.

The surveys of the LHON families from different populations have revealed that only 50 to 72% of the families harbor the ND4/11778 mutation [3–5], indicating genetic heterogeneity of the disease. Recently, another point mutation was detected in 3 out of 21 independent Finnish LHON families without the ND4/11778 mutation, but in none of the 60 controls [6]. A missense G to A mutation at nt3460 in the ND1 gene was detected in these families. This mutation converts an alanine to a threonine at position 52 of the ND1 protein. The base change abolishes an *Aha*II restriction site facilitating an easy detection of the mutation. Subsequently, the ND1/3460 mutation has been detected in several American, British, and Danish families (Ref. 7; Norby, S., personal communication).

The ND1 gene is evolutionarily the most conserved of the ND genes suggesting that its product is intimately involved in the enzyme's function [8]. The ND1 protein is extremely rich in hydrophobic amino acids and contains relatively few hydrophilic regions. The G-to-A transition at nt 3460 leads to a biochemically radical change of a hydrophobic alanine to a hydrophilic threonine in the NH_2 terminus of the polypeptide. The evolutionary conservation at codon 52 also provides evidence for the functional significance of the mutation in the pathogenesis of LHON.

About 2/3 of the Finnish LHON families harbor either the ND4/11778 or the ND1/3460 mutation [6]. In the survey by Howell et al. [7] the same mutation was detected in 5 out of 11 non-ND4/11778 LHON families. The ND4/11778 and the ND1/3460 mutations are thus the prevalent mutations associated with LHON. However, there is still a substantial number of LHON families in which the molecular basis of the disease is unknown. Several alternative candidate mutations have recently been reported in the LHON families [9,10]. These new mutations were found in the ND2 and ND5 genes of Complex I and in the cytochrome *b* gene of Complex III. Each of the mutations alters a conserved amino acid. With the exception of one, the ND2 and ND5 mutations were also found in controls, though at a very low frequency. Subsequent studies have shown that most of these mutations are also detected in the families with the ND4/11778 mutation (unpublished data). Clearly, the association

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of the ND2 and ND5 mutations to the pathogenesis of LHON needs to be further evaluated.

The finding that several mtDNA missense mutations are accumulated in the same maternal lineages have led Brown et al. [10] to suggest that the mutations may act synergistically to result in LHON, each increasing the probability of blindness. Further, it was postulated that the involvement of both Complex I and Complex III mutations in LHON indicates that the clinical manifestations of the disease are related to an overall reduction in cellular energy production rather than to a defect in a specific enzyme.

Heteroplasmy

Mitochondrial DNA replicates within the mitochondrion, and the mitochondria divide asymmetrically without regard to mitochondrial DNA content or timing of replication. The presence of mutated and wild-type mtDNA in the same individual, i.e. heteroplasmy, has been reported both in patients with mitochondrial myopathy and with LHON (e.g., Refs. 11,12). The relative proportions of mtDNA types can shift markedly from one generation to another. Varying amounts of normal and mutated mtDNA in different tissues of the same individual have also been found. It has been suggested that the proportion of mutated mtDNA in the optic nerves or their vasculature might in part explain the variation of expression of optic atrophy found in the families with LHON [13]. However, the tissues mainly affected by the disease – the optic nerve and the retina – are not available for analyses, which hampers the estimation of the threshold level of mutant mtDNA necessary for the expression of LHON.

Biochemistry of LHON

Although several lines of indirect evidence strongly suggest a pathogenic role of the ND4/11778 and ND1/3460 mutations, little is still known about the energy metabolism in LHON and its possible role in the pathogenesis of this disease. Parker et al. [14] have reported reduced NADH:ubiquinone reductase activity in an atypical LHON family with severe neurologic symptoms, which was later shown to have the ND1/4160 mutation [15].

Recently, Majander et al. [16] studied Complex I electron transfer activity in mitochondria and inner mitochondrial membranes derived from peripheral lymphoblast cell lines of patients with ND1/3460 and ND4/11778 mutations. The ND1 mutation exhibited a 80% reduction in rotenone-sensitive and ubiquinone-dependent electron transfer activity, whereas the proximal NADH dehydrogenase activity of the complex was unaffected. This is in accordance with the suggestion that the ND1 subunit interacts with rotenone and ubiquinone. In contrast, the ND4 mutation had no effect on electron transfer activity of the complex in inner mitochondrial membrane preparations; the K_m for NADH in NADH dehydrogenase activity was also unaffected. However, in intact mitochondria with the ND4 mutation the rates of oxidation of NADH-dependent substrates, but not of succinate, were significantly decreased. This suggests that the ND4 subunit might be involved in specific aggregation of NADH-dependent dehydrogenases and Complex I, which may result in fast electron transfer from the former to the latter. Recently, Larsson et al. [17] have reported unaffected electron transfer activity of Complex I itself in isolated mitochondria from muscles of LHON patients, but lowered activities of oxidation of NADH-linked substrates. This finding is in good agreement with the data presented by Majander et al. [16].

In conclusion, while our knowledge of the molecular genetics in LHON is continually increasing, specific information about the energy metabolism and the pathogenesis of the disease is still needed.

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References

1 Nikoskelainen, E.K., Savontaus, M.-L., Wanne, O.P., Katila, M.J. and Nummelin, K.U. (1987) Arch. Ophthalmol. 105, 665–671.
2 Wallace, D.C., Singh, G., Lott, M.T., Hodge, J.A., Schurr, T.G., Lessa, A.M.S., Elsas, L.J. and Nikoskelainen, E.K. (1988) Science 242, 1427–1430.
3 Vilkki, J, Savontaus, M-L. and Nikoskelainen, E.K. (1989) Am. J. Hum. Genet. 45, 206–211.
4 Holt, I.J., Miller, D.H. and Harding, A.E. (1989) J. Med. Genet. 26, 739–743.
5 Poulton, J., Deadman, M.E., Bronte-Stewart, J., Foulds, W.S. and Gardiner, R.M. (1991) J. Med. Genet. 28, 765–770.
6 Huoponen, K., Vilkki, J., Aula, P., Nikoskelainen, E.K. and Savontaus, M.-L. (1991) 48, 1147–1153.
7 Howell, N., Bindoff, L.A., McCullough, D.A., Kubacka, I., Poulton, J., Mackey, D., Taylor, L. and Turnbull, D.M. (1991) Am. J. Hum. Genet. 49, 939–950.
8 Friedrich, T., Strohdeicher, M., Hofhaus, G., Preis, D., Sahm, H. and Weiss, H. (1990) FEBS Lett. 265, 37–40.
9 Johns, D.R. and Berman, J. (1991) Biochem. Biophys. Res. Commun. 174, 1324–1330.
10 Brown, M.D., Voljavec, A.S., Lott, M.T., Torroni, A., Yang, C.-C. and Wallace D.C. (1992) Genetics 130, 163–173.
11 Vilkki, J., Savontaus, M.-L. and Nikoskelainen, E.K. (1990) Am. J. Hum. Genet. 47, 95–100.
12 Zeviani, M., Moraes, C.T., DiMauro, S., Nakase, H., Bonilla, E., Schon, E.A. and Rowland, L.P. (1988) Neurology 38, 1339–1346.
13 Harding, A.E. (1991) Trends Neurosci. 14, 132–138.
14 Parker, W.D, Oley, C.A. and Parks, J.K. (1989) N. Engl. J. Med. 320, 1331–1333.
15 Howell, N. and McCullough, D. (1991) Am. J. Hum. Genet. 48, 1211–1213.
16 Majander, A., Huoponen, K., Savontaus, M.-L., Nikoskelainen, E.K and Wikström, M. (1991) FEBS Lett. 292, 289–292.
17 Larsson, N.-G., Andersen, O., Holme, E., Oldfors, A. and Wahlström, J. (1991) Ann. Neurol. 30, 701–708.