Review article

Leber hereditary optic neuropathy - a disease with a known molecular basis but a mysterious mechanism of pathology

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Abstract. Leber hereditary optic neuropathy is a maternally inherited type of blindness caused by degeneration of the optic nerve. It is caused by point mutations in mitochondrial DNA. Like in other mitochondrial diseases, its penetrance and inheritance is complicated by heteroplasmy, tissue distribution, and the bottleneck phenomenon in oocyte maturation. On the cellular level, the mechanism of the disease development is still mysterious. Currently three theories of pathomechanism of LHON are considered: biochemical, ROS (reactive oxygen species) and apoptotic.

Key words: LHON, mitochondrial diseases, optic atrophy.

Introduction

Leber hereditary optic neuropathy (LHON) is the most frequent mitochondrial disease in Caucasian populations. It is diagnosed in 1 : 25,000 births in northern England (MAN et al. 2002) and 1 : 40,000 in Finland (HUOPONEN 2001). It is characterized by a bilateral acute or subacute loss of vision caused by degeneration of the optic nerve, observed mostly in young men. For the first time it was described by Teodor Leber in 1871. Although this maternally inherited disease caused by mitochondrial DNA mutations has been known for quite a long time,

there are still many questions to answer, connected with the inheritance, penetrance and mechanism of disease development.

Clinical manifestations

One of the first symptoms of LHON is a visual field loss with an enlarging blind spot progressively becoming a centrocaecal scotoma. Color vision is also lost early. Loss of vision occurs simultaneously in both eyes or one eye precedes the other, with a time difference up to more than 6 months in rare cases. The disease is acute when the maximal vision loss takes less than one month, whereas subacute when 5 to 8 months, but it can progressively take even 2 years. The mean age of onset is between 27 and 34 years, but the range is between 1 and 70 years (OMIM).

Generally vision loss is painless, although some patients suffer pain in the affected eye or during eye movement. In some cases the Uthoff phenomenon (vision worsening in consequence of physical effort or rise of body temperature) is observed (HUOPONEN 2001).

In fundoscopy (examination with an ophthalmoscope), circumpapillary teleangiectatic microangiopathy with swelling of a peripapillary retinal nerve fiber is found. In an early vision loss no disturbance is seen in visual evoked potentials (VEPs), but later changes in amplitude or delay in VEPs can be observed. Computer tomography of the brain and standard magnetic resonance imaging of the optic nerve is normal, but scans using short-time inversion recovery (STIR) signal show changes representing gliosis (CHALMERS, SCHAPIRA 1999).

In most of the cases, a loss of vision is the only symptom of the disease, but several families have been described with accompanying neurological symptoms, like bilateral lesions of the putamen on MRI (magnetic resonance image), tremor, ataxia, dystonia, corticospinal tract dysfunction, deafness, skeletal deformities (OMIM; NIKOSKELAINEN et al. 1995).

Frequently the mitochondrial mutations generally responsible for Leber syndrome are connected with other neurodegenerative diseases, especially with multiple sclerosis (MS), in which visual problems are often the very first symptoms signalling the onset of the disease. There are data supporting that theory (VANOPDENBOSCH et al. 2000) as well as rejecting it (HWANG et al. 2001, LEUZZI et al. 1997, NISHIMURA et al. 1995)

The first mitochondrial mutation in LHON was discovered in 1988 by Wallace (WALLACE et al. 1988).

Biochemical characteristics of cells with LHON mutations

In cells harboring different LHON mutations, alterations in respiratory chain functions are frequently observed, but their intensity varies depending on cell

type, and in some studies no reduction in respiratory parameters is found (VERGANI et al. 1995). As most of LHON mutations are located in ND (NADH dehydrogenase) genes, complex I deficiency is detected. To test the effects of these mutations in a common nuclear background, cybrids are used. These are cytoplasmic hybrids which are obtained after fusion of Rho0 cells – without mtDNA and enucleated cells. BROWN et al. (2000) showed that in cybrids made by fusion with LHON cells and in lymphoblasts from LHON patients, complex I activity is reduced by 79% for the G3460A mutation, 20% for the G11778A mutation, while no reduction was observed for the T14484C mutation. For all three mutations maximal respiration rate was lowered, but no difference between cell lines with mutation from affected and unaffected individuals was observed (BROWN et al. 2000). The last finding leads to the question about the background of the disease. It is possible that respiratory consequences of lowered activity of complex I are not the direct cause of LHON.

It seems that all LHON mutations in ND genes affect the interaction between complex I and ubiquinone substrates and inhibitors at the quinone/quinol binding site (MAJANDER et al. 1991).

Genetic background

More than 18 mitochondrial mutations are supposed to be responsible for disease development. Depending on the source, 5 or more of them are considered as primary mutations, which means that they are real causes of the loss of vision (OMIM, MITOMAP).

All known mitochondrial mutations causing LHON map to genes encoding protein subunits, mostly NADH dehydrogenase, but a few mutations in other protein-coding genes are probably also involved (i.e. CO3, ATP6, Cytb). The most frequent three mutations are: G3460A, G11778A, and T14484C (Figure 1). Altogether they are responsible for about 90 % of Leber cases. G11778A is diagnosed in about 69 %, G3460A in 13% and T14484C in 14% of patients in the Caucasian population (HUOPONEN 2001). Beside these three very frequent mutations six others, considered as primary, were detected in the ND6 gene which seems to be a hot spot for LHON mutations (CHINNERY et al. 2001b).

The other mutations are the so-called secondary mutations, and their role in the disease is not clear (OMIM). Presence of two secondary mutations T4216C and G13708A does not further impair mitochondrial oxygen metabolism in cells with the G11778A mutation (LODI et al. 2000). They could influence disease progression or only be polymorphic haplogroup markers (see later) – a sign of a common ancestry that does not affect the disease process (NIKOSKELAINEN et al. 1996).

Primary mutations can be divided into mild and severe ones. The former lead to a quick and complete loss of vision, whereas patients developing LHON in con-

sequence of mild mutations can retain light perception and the progress of the disease can be slower. The most severe one is the G14459A mutation found in Russian and Hispanic families, causing not only LHON but also other neurological diseases. Considering the three most frequent mutations, G11778A is the most severe and the most common one, responsible for about 50% of LHON cases in Europe and even 95% in Asia. Next is the G3460A mutation, responsible for about 35% of European cases, and the least severe is the T14484C mutation diagnosed in about 20% of European patients (OMIM).

As all mitochondrial diseases, LHON is maternally inherited, but not all individuals carrying the mutation express the disease. Penetrance is about 60% for men and 20% for women. In the Caucasian population another interesting phe-
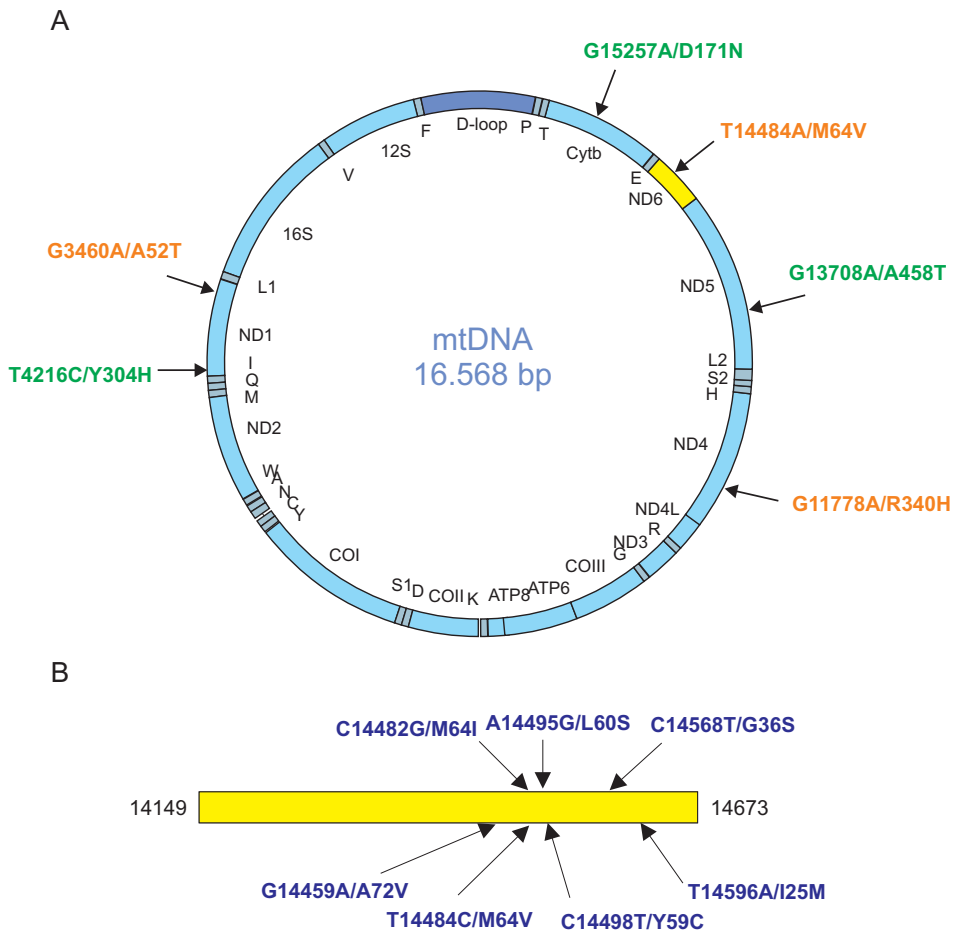

Figure 1. Mutations causing LHON

A = Three common primary mutation (in orange) and the most common secondary mutations (in green).
B = Seven primary mutations in ND6 gene

nomenon is observed: more males than females are affected. For example, in the Finnish population 80% of Leber patients are males (HUOPONEN 2001, HUOPONEN et al. 1993). The reason for both observations is still unknown, but strongly suggests that the mitochondrial DNA mutation is not sufficient to cause the disease and the influence of nuclear genes, probably located on chromosome X, is needed (BU, ROTTER 1991). In several publications the existence of an X-linked gene near the DXS7 locus, cooperating in expression of the disease, was proposed (VILKKI et al. 1991), but linkage analysis in British, Italian and German LHON families did not give significant results, and excluded this suspicion (CHALMERS et al. 1996, SWEENEY et al. 1992). Additionally, PEGORARO (2003) found no evidence of aberrations in X-chromosome inactivation.

The last genetic factor suspected of modulating Leber syndrome is the haplogroup. Mitochondrial DNA variants are grouped in a way reflecting the evolutionary history of mtDNA differentiation. They are called haplogroups, and mtDNA types belonging to the same haplogroup are characterized by several common polymorphisms. Haplogroups H, I, J, K, T, U, W, V, X cover most of the European mtDNAs. Analysing haplogroups of patients with Leber mutations, a higher frequency of haplogroup J was observed for mutations G11778A and T14484C in several studies (HUOPONEN 2001, TORRONI et al. 1997). It was proposed that polymorphisms characteristic for that haplogroup in fact influence the respiratory chain activity and cooperate in disease formation, but as was mentioned earlier, the presence of mutations T4216C/ND1 and G13708A/ND5 characteristic for haplogroup J does not further impair the respiratory chain activity. On the other hand, lower penetrance was observed in carriers of the T14484C mutation not belonging to haplogroup J (HOWELL et al. 2003). Another possibility is that this phenomenon can be caused by a founder effect. This seems very likely for the T14484C mutation, where about 75% of cases belong to haplogroup J. We analysed haplogroups for 8 patients with the G11778A mutation and none of them belonged to haplogroup J (MROCZEK-TONSKA et al. 2002); the only patient who belonged to haplogroup J was one with a T14484C mutation (unpublished). Because of the small number of samples the obtained data are not statistically significant but it is possible that Polish patients differ from the other European G11778A mutation carriers. The role of polymorphisms of haplogroup J in LHON is still unclear.

Heteroplasmy

Heteroplasmy is the existence of more than one type of mitochondrial DNA in one cell. It is common in mitochondrial diseases where the patients carry both mutated and unmutated DNA. The same phenomenon is observed in LHON, but not for all mutations. Generally, more severe mutations are more often heteroplasmic. It seems possible that such severe mutations could be lethal in

the homoplasmic state. The other, less severe mutations are present in the homoplasmic state. But this is not the rule: in some cases even a severe mutation, like G11778A, can exist in a homoplasmic or almost homoplasmic state (more than 95% of mutated DNA) (MROCZEK-TONSKA et al. 2002), and mild mutations can be heteroplasmic. There are several other problems connected with heteroplasmy – tissue distribution and inheritance (discussed later). For example, the level of G11778A mutations observed in affected and unaffected members of the same family can be similar. Such an observation can be explained by the influence of nuclear genes (discussed above) and the differences in tissue distribution. Leber disease is molecularly diagnosed by using DNA isolated from peripheral blood, which is the easiest way but can lead to imprecise results, because pathologic changes are observed exclusively in the optic nerve, and the mutation level can differ between the optic nerve and blood leukocytes. In affected individuals the level of LHON mutations in the optic nerve can be higher than in unaffected ones with an identical level in blood (CHALMERS, SCHAPIRA 1999, HUOPONEN 2001). In this very confusing picture some rules can be observed. After analysing 17 independent families with the G11778A mutation, CHINNERY (2001a) found that in males the frequency of blindness was related to the mutation level in blood and mothers with a higher mutation load more often had affected children.

Inheritance of LHON mutations in families

All discoveries made for other mitochondrial diseases are true for LHON. Children of mothers with a low Leber mutation level can inherit similar or different mutation levels, varying from 0 to 100% (CHINNERY et al. 2001a). Even if the mother has a homoplasmic mutation, it is essentially impossible to foresee the mutation level in her children. This is explained again by the tissue distribution and the phenomenon called bottleneck, taking place in a very early stage of oocyte development. The number of mtDNA molecules decreases to several hundred and then grows to about a hundred thousand in the mature oocyte. In consequence, oocytes can differ in the mutation level because of a kind of genetic drift taking place in the oocyte during development (JENUTH et al. 1996).

LHON mutations in the optic nerve

The limitation of symptoms almost exclusively to the optic nerve is very surprising. Even if the mutation level is very high, in other tissues there are no additional effects. To resolve this problem, WONG (2002) constructed cybrids with the LHON mutations, based on neuronal NT2 cells. Before differentiation, LHON cybrids and control cells behaved in a similar way: the amount of mtDNA and various mitochondrial parameters did not show significant differences. The picture

changed after differentiation. Although parameters like mitochondrial membrane potential and ability to reduce Almar blue did not change, fewer LHON cells could be observed and ROS production was increased. A hypothesis alternative to the bioenergetic one may be that the optic nerve is more sensitive to ROS, whose production is higher in LHON than in normal cells.

LHON and apoptosis

All the information presented above indicates that the reason for Leber syndrome is known – it is caused by mitochondrial mutations – but little is known about how mitochondrial mutations lead to disease development. In the last years, an explanation different from a direct loss of respiratory chain activity appeared. Mitochondria in cells are not only energy generators. It is even possible that energy production is not the most important mitochondrial function – cultures of cells without mtDNA, thus without a working respiratory chain (Rho0 cells), supplemented with pyruvate and uridine, can grow using energy obtained only from glycolytic processes. Mitochondria also play a role in fatty acid β-oxidation, metabolism of some amino acids, and apoptosis. Moreover, the Fas apoptotic pathway goes through mitochondria. Activation of cell membrane receptor Fas by a Fas ligand causes caspase-8 cleavage. Caspase-8 cuts Bid protein (a member of Bcl2 proapoptotic proteins), causing its translocation to the outer mitochondrial membrane and release of cytochrome c. In the cytoplasm, cytochrome c binding with cytosolic protein Apaf-1 causes amplification of the apoptotic signal, by activating the caspase cascade through caspase-9. The last years have brought more and more information about new proteins responsible for programmed cell death present in mitochondria (WANG 2001).

There are several hints that pathology of the Leber syndrome may be connected with apoptosis. First, degeneration of the optic nerve, leading to the loss of vision, seems to appear in an apoptotic way (HOWELL 1998). Second, studies of cybrids with G11778A and G3460A mutations show a higher sensibility for Fas-induced apoptosis, in comparison to analogous cells without LHON mutation (DANIELSON et al. 2002). That phenomenon can be explained by alterations in complex I. It is known that complex I and ubiquinone analogs play a regulatory role in the opening of the mitochondrial transition pore. Also metabolic stress produced by culture on galactose media (where cells have to rely only on oxygen respiration) lead to cell death and cybrids with the above mutations quickly switch to the apoptotic pathway (GHELLI et al. 2003). All these results suggest that sensitizing to apoptosis may be the actual role of LHON mutations in the disease development.

REFERENCES

BROWN M.D., TROUNCE I.A., JUN A.S., ALLEN J.C., WALLACE D.C. (2000). Functional analysis of lymphoblast and cybrid mitochondria containing the 3460, 11778, or 14484 Leber's hereditary optic neuropathy mitochondrial DNA mutation. J. Biol. Chem. 275: 39831-39836.

BU X.D., ROTTER J.I. (1991). X chromosome-linked and mitochondrial gene control of Leber hereditary optic neuropathy: evidence from segregation analysis for dependence on X chromosome inactivation. Proc. Natl. Acad. Sci. USA 88: 8198-8202.

CHALMERS R.M., DAVIS M.B., SWEENEY M.G., WOOD N.W., HARDING A.E. (1996). Evidence against an X-linked visual loss susceptibility locus in Leber hereditary optic neuropathy. Am. J. Hum. Genet. 59: 103-108.

CHALMERS R.M., SCHAPIRA A.H. (1999). Clinical, biochemical and molecular genetic features of Leber's hereditary optic neuropathy. Biochim. Biophys. Acta 1410: 147-158.

CHINNERY P.F., ANDREWS R.M., TURNBULL D.M., HOWELL N.N. (2001a). Leber hereditary optic neuropathy: Does heteroplasmy influence the inheritance and expression of the G11778A mitochondrial DNA mutation? Am. J. Med. Genet. 98: 235-243.

CHINNERY P.F., BROWN D.T., ANDREWS R.M., SINGH-KLER R., RIORDAN-EVA P., LINDLEY J., APPLEGARTH D.A., TURNBULL D.M., HOWELL N. (2001b). The mitochondrial ND6 gene is a hot spot for mutations that cause Leber's hereditary optic neuropathy. Brain 124: 209-18.

DANIELSON S.R., WONG A., CARELLI V., MARTINUZZI A., SCHAPIRA A.H., CORTOPASSI G.A. (2002). Cells bearing mutations causing Leber's hereditary optic neuropathy are sensitized to Fas-induced apoptosis. J. Biol. Chem. 277: 5810-5815.

GHELLI A., ZANNA C., PORCELLI A.M., SCHAPIRA A.H., MARTINUZZI A., CARELLI V., RUGOLO M. (2003). Leber's hereditary optic neuropathy (LHON) pathogenic mutations induce mitochondrial-dependent apoptotic death in transmitochondrial cells incubated with galactose medium. J. Biol. Chem. 278: 4145-4150.

HOWELL N. (1998). Leber hereditary optic neuropathy: respiratory chain dysfunction and degeneration of the optic nerve. Vision Res. 38: 1495-1504.

HOWELL N., HERRNSTADT C., SHULTS C., MACKEY D.A. (2003). Low penetrance of the 14484 LHON mutation when it arises in a non-haplogroup J mtDNA background. Am. J. Med. Genet. 119A: 147-151.

HUOPONEN K. (2001). Leber hereditary optic neuropathy: clinical and molecular genetic findings. Neurogenetics 3: 119-125.

HUOPONEN K., LAMMINEN T., JUVONEN V., AULA P., NIKOSKELAINEN E., SAVONTAUS M.L. (1993). The spectrum of mitochondrial DNA mutations in families with Leber hereditary optic neuroretinopathy. Hum. Genet. 92: 379-384.

HWANG J.M., CHANG B.L., PARK S.S. (2001). Leber's hereditary optic neuropathy mutations in Korean patients with multiple sclerosis. Ophthalmologica 215: 398-400.

JENUTH J.P., PETERSON A.C., FU K., SHOUBRIDGE E.A. (1996). Random genetic drift in the female germline explains the rapid segregation of mammalian mitochondrial DNA. Nat. Genet. 14: 146-151.

LEUZZI V., CARDUCCI C., LENZA M., SALVETTI M., RISTORI G., DI GIOVANNI S., TORRONI A. (1997). LHON mutations in Italian patients affected by multiple sclerosis. Acta. Neurol. Scand. 96: 145-148.

LODI R., MONTAGNA P., CORTELLI P., IOTTI S., CEVOLI S., CARELLI V., BARBIROLI B. (2000). 'Secondary' 4216/ND1 and 13708/ND5 Leber's hereditary optic neuropathy mitochondrial DNA mutations do not further impair in vivo mitochondrial oxidative metabolism when associated with the 11778/ND4 mitochondrial DNA mutation. Brain 123: 1896-1902.

MAJANDER A., HUOPONEN K., SAVONTAUS M.L., NIKOSKELAINEN E., WIKSTROM M. (1991). Electron transfer properties of NADH:ubiquinone reductase in the ND1/3460 and the ND4/11778 mutations of the Leber hereditary optic neuroretinopathy (LHON). FEBS Lett. 292: 289-292.

MAN P.Y., TURNBULL D.M., CHINNERY P.F. (2002). Leber hereditary optic neuropathy. J. Med. Genet. 39: 162-169.

MITOMAP: http://www.mitomap.org/

MROCZEK-TONSKA K., RATAJSKA D., GUILLOT C., SASIADEK M., AMBROZIAK A., LUBOS L., BARTNIK E. (2002). Heteroplasmy analysis in the Polish patients with 11778A mutation responsible for Leber hereditary optic neuropathy. Acta Biochim. Pol. 49: 257-262.

NIKOSKELAINEN E.K., HUOPONEN K., JUVONEN V., LAMMINEN T., NUMMELIN K., SAVONTAUS M.L. (1996). Ophthalmologic findings in Leber hereditary optic neuropathy, with special reference to mtDNA mutations. Ophthalmology 103: 504-514.

NIKOSKELAINEN E.K., MARTTILA R.J., HUOPONEN K., JUVONEN V., LAMMINEN T., SONNINEN P., SAVONTAUS M.L. (1995). Leber's "plus": neurological abnormalities in patients with Leber's hereditary optic neuropathy. J. Neurol. Neurosurg. Psychiatry 59: 160-164.

NISHIMURA M., OBAYASHI H., OHTA M., UCHIYAMA T., HAO Q., SAIDA T. (1995). No association of the 11778 mitochondrial DNA mutation and multiple sclerosis in Japan. Neurology 45: 1333-1334.

OMIM: http://www.ncbi.nlm.nih.gov/omim/

PEGORARO E., VETTORI A., VALENTINO M.L., MOLON A., MOSTACCIUOLO M.L., HOWELL N., CARELLI V. (2003). X-inactivation pattern in multiple tissues from two Leber's hereditary optic neuropathy (LHON) patients. Am. J. Med. Genet. 119A: 37-40.

SWEENEY M.G., DAVIS M.B., LASHWOOD A., BROCKINGTON M., TOSCANO A., HARDING A.E. (1992). Evidence against an X-linked locus close to DXS7 determining visual loss susceptibility in British and Italian families with Leber hereditary optic neuropathy. Am. J. Hum. Genet. 51: 741-748.

TORRONI A., PETROZZI M., D'URBANO L., SELLITTO D., ZEVIANI M., CARRARA F., CARDUCCI C., LEUZZI V., CARELLI V., BARBONI P., DE NEGRI A., SCOZZARI R. (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.

VANOPDENBOSCH L., DUBOIS B., D'HOOGHE M.B., MEIRE F., CARTON H. (2000). Mito-
    chondrial mutations of Leber's hereditary optic neuropathy: a risk factor for multiple
    sclerosis. J. Neurol. 247: 535-543.
VERGANI L., MARTINUZZI A., CARELLI V., CORTELLI P., MONTAGNA P.,
    SCHIEVANO G., CARROZZO R., ANGELINI C., LUGARESI E. (1995). MtDNA muta-
    tions associated with Leber's hereditary optic neuropathy: studies on cytoplasmic hy-
    brid (cybrid) cells. Biochem. Biophys. Res. Commun. 210: 880-888.
VILKKI J., OTT J., SAVONTAUS M.L., AULA P., NIKOSKELAINEN E.K. (1991). Optic atro-
    phy in Leber hereditary optic neuroretinopathy is probably determined by
    an X-chromosomal gene closely linked to DXS7. Am. J. Hum. Genet. 48: 486-491.
WALLACE D.C., SINGH G., LOTT M.T., HODGE J.A., SCHURR T.G., LEZZA A.M.,
    ELSAS L.J., 2ND, NIKOSKELAINEN E.K. (1988). Mitochondrial DNA mutation asso-
    ciated with Leber's hereditary optic neuropathy. Science 242: 1427-1430.
WANG X. (2001). The expanding role of mitochondria in apoptosis. Genes Dev. 15:
    2922-2933.
WONG A., CAVELIER L., COLLINS-SCHRAMM H.E., SELDIN M.F., MCGROGAN M.,
    SAVONTAUS M.L., CORTOPASSI G.A. (2002). Differentiation-specific effects
    of LHON mutations introduced into neuronal NT2 cells. Hum. Mol. Genet. 11:
    431-438.