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ORIGINAL INVESTIGATION

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Novel mtDNA mutations and oxidative phosphorylation dysfunction in Russian LHON families

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Abstract Leber's hereditary optic neuropathy (LHON) is characterized by maternally transmitted, bilateral, central vision loss in young adults. It is caused by mutations in the mitochondrial DNA (mtDNA) encoded genes that contribute polypeptides to NADH dehydrogenase or complex I. Four mtDNA variants, the nucleotide pair (np) 3460A, 11778A, 14484C, and 14459A mutations, are known as "primary" LHON mutations and are found in most, but not all, of the LHON families reported to date. Here, we report the extensive genetic and biochemical analysis of five Russian families from the Novosibirsk region of Siberia manifesting maternally transmitted optic atrophy consistent with LHON. Three of the five families harbor known LHON primary mutations. Complete sequence analysis of proband mtDNA in the other two families has revealed novel complex I mutations at nps 3635A and 4640C, respectively. These mutations are homoplasmic and have not been reported in the literature. Biochemical analysis of complex I in patient lymphoblasts and transmitochondrial cybrids demonstrated a respiration defect with complex-I-linked substrates, although the specific activity of complex I was not reduced. Overall, our data suggests that the spectrum of mtDNA mutations associated with LHON in Russia is similar to that in Europe and North America and that the np 3635A and 4640C mutations may be additional mtDNA complex I mutations contributing to LHON expression.

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Introduction

Leber's hereditary optic neuropathy (LHON) is characterized by the maternal transmission of acute or subacute, progressive, and bilateral loss of central vision attributable to optic nerve dysfunction (Newman et al. 1991; Harding et al. 1995). In 1988, LHON was shown to result from a G to A mutation at nucleotide position (np) 11778 of mitochondrial DNA (mtDNA; Wallace et al. 1988). Since then, four other mtDNA variants, 3460A, 14484C, 14459A, and 14495G, have been identified that cause familial LHON (Howell et al. 1991; Huoponen et al. 1991; Johns et al. 1992; Chinnery et al. 2001). Although nearly 20 mtDNA mutations have been associated with LHON, the 3460A, 11778A, 14484C, and 14459A mutations have been designated "primary" LHON mutations because they are significant risk factors for blindness. Primary LHON mutations share certain genetic features, such as absence in control individuals, occurrence in multiple independent LHON families, and a very rare incidence of co-occurrence within families (Brown and Wallace 1994; Brown et al. 1995; Howell et al. 1995; Howell 1998). "Secondary" LHON mtDNA mutations are more ambiguous than primary mutations with respect to LHON etiology but include the intriguing European haplogroup J-associated mutations 4216C and 13708A, which are consistently found in high frequencies in LHON patients but are also present in European controls. With the exception of the 14459A mutation, which can also result in early onset dystonia (Jun et al. 1994; Shoffner et al. 1995) or Leigh's syndrome (Kirby et al. 2000), optic atrophy is typically the only clinical manifestation of LHON. Overall, these mutations account for more than 90% of all reported LHON cases, although multi-generational families do exist in which a known primary mutation is not present, suggesting further genetic heterogeneity (Brown et al. 1995).

It is noteworthy that all primary LHON mtDNA mutations alter polypeptides that are components of NADH dehydrogenase or complex I. Complex I is a large multi-subunit enzyme comprised of seven mtDNA-encoded (ND1, 2, 3, 4, 4L, 5, and 6) and roughly 35 nuclear-encoded polypeptides (Wallace et al. 1996). In oxidative phosphorylation (OXPHOS), electrons enter the mitochondrial electron transport chain from NADH $+H^+$ via complex I. In complex I, the electrons traverse a flavin mononucleotide, 5–7 iron-sulfur centers, and ultimately reduce ubiquinone to ubiquinol. From ubiquinol, the electrons are transferred to complex III (ubiquinol:cytochrome c oxidoreductase, [EC 1.10.2.2]), then to cytochrome c, next to complex IV (cytochrome c oxidoreductase, [EC 1.9.3.1]), and finally to atomic oxygen. Electrons can also be donated from succinate to complex II, which then reduces coenzyme Q (CoQ_{10}), by-passing complex I. As electrons traverse complexes I, III, and IV, protons are translocated from the matrix to the inner membrane space creating an electrochemical gradient ($\Delta\Psi$). $\Delta\Psi$ is utilized by complex V (ATP synthase, [EC 3.6.1.34]) to condense ADP and inorganic phosphate to ATP.

Recent work has demonstrated that the primary LHON mutations cause a partial complex I deficiency, but to varying degrees (for a review, see Brown 1999). The 3460A and 14459A mutations result in a marked reduction of complex I specific activity in submitochondrial particles, and the 3460A and 11778A mutations exhibit moderate reductions in complex-I-linked respiration rates. The 14484C variant results in only a minimal perturbation of complex I function. Thus, the in vitro biochemical signatures of the LHON mutants varies, adding complexity to the effort to understand the underlying pathophysiological mechanism of this disease (Howell 1998; Brown 1999; Brown et al. 2000).

Although the spectrum of bioenergetic disease is substantial and growing, reports of mitochondrial disease in Russia are very rare (Kniazev et al. 2000). In the present study, we have used restriction fragment length polymorphism analysis and whole genome sequencing to identify pathogenic mtDNA mutations in five Siberian LHON families. We have found that three of the families harbor known primary LHON mutations, whereas the other two contain novel mutations in complex I genes. To determine the biochemical effects of the two novel mutations, we have performed respiration and enzymological analyses of the proband's lymphoblasts and of transmitochondrial cybrids, confirming the presence of complex I defects. Therefore, LHON in Russia is associated with both classical and novel LHON mtDNA mutations.

Materials and methods

LHON patients and families

Five Caucasian Siberian families of Slavic origin exhibited maternal transmission of bilateral optic neuropathy (Fig. 1, Table 1). All five families demonstrated clinical characteristics consistent with the diagnosis of LHON (Newman et al. 1991; Riordan-Eva et al. 1995). No other ophthalmologic cause of visual loss was apparent.

The age of onset ranged from 12 to 38 years, with a mean of 23 years. Males were affected more commonly than females, except in Family I, in which there were one affected male and three affected females. Visual loss was typically bilateral, painless and sudden, with an interval between loss of central vision in each eye of 0–6 months. Each eye progressively worsened for up to 8 months, typically over 2–4 months. End-point visual acuities were poor, usually well within the range of legal blindness. Affected individuals in Families D and I showed no significant visual recovery. One male member of Family S who had visual loss at age 27 subjectively believed that his vision improved one year after onset and then worsened, but this was not well documented. Two male members each from Families E and M had some late visual recovery. The age of onset of initial visual loss was 15–32 years in the individuals from Family E, and 12–30 years in the individuals from Family M. The proband from Family M with visual loss at age 14 had subsequent worsening after documentation of improvement. Abnormal electrocardiograms were documented in maternal members of all the families, except for Family E. In Family D, Wolf-Parkinson-White pre-excitation syndrome was documented in one affected male member, and a possible arrhythmia was documented in another affected male member. Non-specific conduction abnormalities were reported in one unaffected female member of Family I. Lown-Ganong-Levin syndrome (a pre-excitation syndrome with PQ-shortening) and sinus bradycardia were documented in two affected males and one unaffected female in Family S. Non-specific conduction abnormalities were present in two affected male members of Family M. Neurologic abnormalities were documented in the maternal lineage of Families I, S, and E. One affected female from Family I had paresis suggestive of multiple sclerosis, and three other unaffected females from the same family had neurologic abnormalities described as "various degrees of ataxia, seizures, paresis and multiple sclerosis". In Family S, there were at least four maternal relatives with neurologic abnormalities, one affected with visual loss and mild ataxia and nystagmus, and three without visual loss but with mild ataxia and nystagmus, two of whom also had myoclonic seizures, one with deafness. In Family E, there was an affected male and an affected female with mild neurologic symptoms suggestive of multiple sclerosis but with normal magnetic resonance imaging. Another affected male from Family E had an Arnold-Chiari malformation thought to be the cause of some mild ataxia, and an additional affected female had partial loss of hearing.

MtDNA analysis

Venous blood samples were obtained from the family members following informed consent. Genomic DNA was isolated from the buffy coat blood fraction or approximately 10^6 Epstein-Barr virus (EBV)-transformed lymphoblasts or cybrids by using Chelex 100 (Cetus, Emeryville, Calif.). We screened for the 4216C, 3460A, 13708A, 11778A, and 14484C LHON mutations by polymerase chain reaction (PCR) amplification followed by mutation-specific restriction endonuclease digestion (Brown et al. 1995). For complete sequence analysis, the mtDNA was PCR-amplified in 2-kb to 3-kb fragments and purified on Centricon 100 columns. The mtDNA was subjected to cycle sequencing by using "BigDye Terminators" (ABI/Perkin-Elmer Cetus), and excess terminators were removed by Centi-Sep columns. Samples underwent gel electrophoresis on an ABI Prism 377 automated DNA sequencer, and trace files were analyzed by using Sequencher (v3.1, GeneCode Corp.) software. The 8551C mutation was confirmed by restriction enzyme digestion, since the 8551C mutation eliminates a *Bsp*HI site at np 8551. The 3635A and the 4640A mutations were confirmed by using mutation-specific restriction enzyme digestion following mismatched-primer PCR amplification of white blood cell mtDNA (Brown et al. 1995).

Cell lines

Lymphoblastoid cell line and transmitochondrial cybrid production have been previously described (Brown et al. 2000). Briefly,

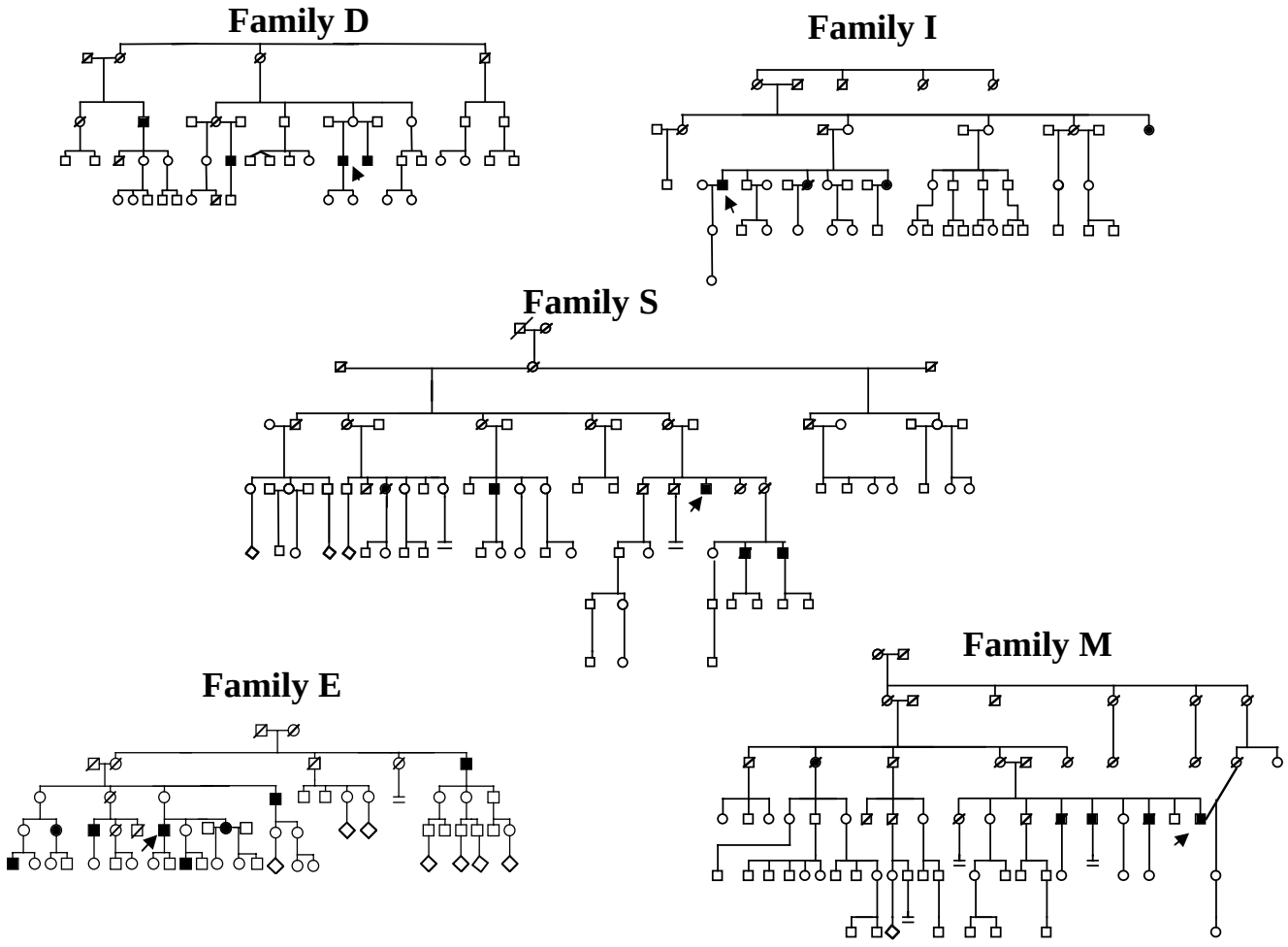

Fig. 1 Five Siberian LHON families of Slavic origin (*arrows* probands)

lymphoblast cell lines were established by EBV transformation of leukocytes isolated from whole blood by Ficoll-Hypaque gradients. All EBV-transformed cell lines were maintained in RPMI 1640 medium (Bio-Whitaker, Walkersville, Md.) supplemented with 15% (vol/vol) heat-inactivated fetal bovine serum (Gibco-BRL life Technologies, Grand Island, N.Y.). Lymphoblast cell lines were ordinarily maintained in culture for 10–20 population doublings prior to mitochondrial isolation. The WAL2A-ρ° line lacks mtDNA, is HPRT-deficient, and is grown in RPMI 1640 medium supplemented with 15% heat-inactivated fetal bovine serum, 4 mg/ml glucose, 50 μg/ml uridine, 1 mM pyruvate (GUP media), plus 1 μg/ml 6-thioguanine (6TG).

Transmitochondrial cybrids were prepared by enucleating EBV-transformed lymphoblastoid cell lines from LHON patients and controls and fusing 2×10^7 mitochondria-containing cytoplasts with the 10^7 WAL2A-ρ° cells by electro-shock (Trounce et al. 1996). Cybrids were selected in 75 ml RPMI 1640 medium supplemented with 10% (vol/vol) dialyzed fetal bovine serum (Gibco-BRL Life Technologies), 2 mg/ml glucose, and 1 μg/m 6TG. Rapid growth of the cybrid cultures was observed 20–28 days post-fusion, and cybrid lines were passaged for 5–10 population doublings prior to mitochondrial isolation. Family E and M lymphoblastoid cell lines and cybrid constructs were homoplasmic.

Table 1 Clinical characteristics of Siberian LHON families (ECG electrocardiogram)

Family	MtDNA mutation	Affected:unaffected ratio (among maternal relatives)	LHON male: LHON female	Age of onset range (mean)	Visual acuity[a]	Recovery	Abnormal ECG[b]	Abnormal neurological[b]
D	11778	4:15 (7M, 8F)	4:0	20–38 (29)	0.03	No	Yes	No
I	11778	4:26 (11M, 15F)	1:3	18–34 (24.5)	0.01	No	Yes	Yes
S	3460	5:36 (17M, 19F)	4:1	21–27 (23.8)	0.04	?	Yes	Yes
E	3635	8:20 (6M, 14F)	3:1	15–33 (22.1)	0.03	Yes	No	Yes
M	4640	5:35 (14M, 21F)	4:1	12–30 (18)	0.05	Yes	Yes	No

[a]Average end-point visual acuity
[b]Among maternally related family members (see Materials and methods)

Mitochondrial isolation, polarography, and OXPHOS enzymology

Procedures for mitochondrial isolation, polarographic analysis, and OXPHOS enzymology of submitochondrial particles have been described (Trounce et al. 1996; Brown et al. 2000). Lymphoblast and cybrid mitochondria were assayed at concentrations ranging from 3.5 to 13 mg protein per ml. For respiration analysis of mitochondria, two polarographic runs were performed for each substrate (malate plus pyruvate, malate plus glutamate, and succinate) involving two additions of ADP (125 nmole ADP for site I and 75 nmole ADP for site II substrates), and a final addition of the OXPHOS uncoupler 2,4-dinitrophenol. All runs were performed with 250–500 μg mitochondrial protein. The respiratory control ratio (RCR) is the mean state III respiration rate divided by the mean state IV respiration rate. All mitochondrial isolates included in this study had to have RCRs of more than 2 as a minimum standard of mitochondrial integrity. The state III ratio is the mean of the state III respiration rates with complex I substrates divided by the mean of the state III respiration rates with the complex II substrate, succinate.

OXPHOS enzyme activities were assayed on sonicated mitochondria and measured spectrophotometrically with a dual-beam UV-visual spectrophotometer (model DW-2000; SLM-Aminco, Urbana, Ill.). For complex I, 30 μg mitochondrial protein was assayed by monitoring the reduction of 10 μM decylubiquinone at 272 nm by 30 μM NADH. Complex I activities were assayed in triplicate. Consistently 90%–100% of the total complex I activity was rotenone-sensitive. Citrate synthase was assayed in duplicate with 15 μg mitochondrial protein.

Table 2. Novel mtDNA sequence changes in Siberian LHON families (*CR* control region, – no amino acid change)

Region/gene	Nucleotide position	Nucleotide change	Amino acid change
Family E[a]			
1. CR	316	Cins	–
2. CR	525	Cdel	–
3. ND1	3635	G to A	Ser to Asn
4. ATP8	8551	T to C	–
ATP6	8551	T to C	Phe to Leu
5. ND3	10362	C to T	–
6. ND4	12070	G to A	–
7. ND5	13899	T to C	–
Family M[b]			
1. ND1	3546	C to A	–
2. ND1	4188	A to G	–
3. ND2	4640	C to A	Ile to Met
4. COI	6359	A to G	–
5. COIII	9656	T to C	–
6. ND5	12720	A to G	–
7. ND5	13743	T to C	–

[a]Previously reported polymorphisms or Cambridge sequencing errors include mutations at nps 73, 150, 152, 263, 295, 489, 523, 1438, 2706, 3107, 3423, 4216, 4769, 4985, 5633, 7028, 7476, 8860, 9559, 10172, 10398, 11251, 11335, 11719, 12612, 13702, 13708, 14199, 14272, 14365, 14368, 15257, 15326, 15452, 15812, 16069, 16126, 16193, 16286, and 16319

[b]Previously reported polymorphisms or Cambridge sequencing errors include mutations at nps 1811, 2706, 3106, 3423, 4769, 4985, 7028, 8860, 9559, 11335, 11467, 11719, 12308, 12372, 13702, 14139, 14199, 14272, 14365, 14368, 15326, 15454, 16343, and 16362

Results

MtDNA analysis

Mutation-specific restriction enzyme digestion of PCR-amplified buffy coat mtDNA demonstrated the presence of primary LHON mutations in the probands of three of the Russian LHON pedigrees. The 11778A mutation was found in the probands of both Family I (homoplasmic) and Family D (heteroplasmic), and Family I was also found to harbor LHON secondary mtDNA mutations 4216C and 13708A. The proband of Family S was heteroplasmic for the 3460A LHON mutation.

Since Families E and M did not contain a known primary LHON mtDNA mutation, the proband mtDNA was sequenced (Table 2). For Family E, we found 48 base changes relative to the standard Cambridge sequence (Anderson et al. 1981), 41 of which were known polymorphisms (http://www.gen.emory.edu/mitomap.html; Kogelnik et al. 1996, 1997). Among the polymorphisms were the np 4216C, 13708A, and 16069T mutations. These variants in part define European mtDNA haplogroup J (Torroni et al. 1996), which has been shown to promote the expression of mild mtDNA mutations (Brown et al. 1995, 1997; Howell et al. 1995; Torroni et al. 1997). Of the seven remaining novel variants, five were silent mutations, and the remaining two were missense mutations at np 3635 (G to A) of the ND1 gene and np 8551 (T to C) of the ATP6/8 gene. The 3635A mutation substitutes an asparagine for a highly conserved serine at ND1

Table 3 Evolutionary conservation of amino acids altered in Siberian LHON families

	Mutation 3635A ND1 gene Amino acid 110 *			Mutation 8551C ATP6 gene Amino acid 9 *			Mutation 4640A ND2 gene Amino acid 57 *		
LHON	LATS	N	LAVY	LFAS	L	IAPT	TEAA	M	KYFL
Human	LATS	S	LAVY	LFAS	F	IAPT	TEAA	I	KYFL
Cow	LAMS	S	LAVY	LFTS	F	ITPV	TEAS	T	KYFL
Mouse	LATS	S	LASY	LFAS	F	ITPT	TEAA	T	KYFL
Frog	LALS	S	LAVY	FFDQ	F	MSPV	IEAS	T	KYFL
Chicken	LAMS	S	LTVY	FFDQ	F	SSPC	IEAT	I	KYFL
Sea urchin	LGLS	S	LSVY	LGQF	F	PETL	VEAD	N	KYFL
Fruit fly	LCCT	S	LGVY	FSVF	F	DESA	TEAS	L	KYFL

*Amino acid changed in LHON patient

Table 4 Respiration rates in Siberian LHON patient and control lymphoblasts and cybrids

Substrate	Individual	Mutation	Mean respiration rates (nmol O/min per mg mitochondrial protein)		ADP/O ratio	State III ratio
			State III	Uncoupled		
Lymphoblasts						
Pyruvate+malate	Patient E	3635A/8551C	61	68	3.1	0.30
	Patient M	4640A	106	111	2.7	0.62
	Controls (*n*=22)	–	140±41	168±60	2.8±0.5	0.78±0.05
Glutamate+malate	Patient E	3635A/8551C	68	56	2.9	0.33
	Patient M	4640A	109	109	2.2	0.64
	Controls (*n*=22)	–	145±41	173±64	2.6±0.6	0.81±0.11
Succinate	Patient E	3635A/8551C	203	213	1.6	–
	Patient M	4640A	171	195	1.3	–
	Controls (*n*=22)	–	179±45	247±96	1.8±0.3	–
Cybrids						
Pyruvate+malate	Patient E	3635A/8551C	41	52	3.3	0.46
	Patient M	4640A	62	76	3.0	0.51
	Controls (*n*=9)	–	97±20	101±20	2.4±0.3	0.76±0.10
Glutamate+malate	Patient E	3635A/8551C	42	42	2.6	0.45
	Patient M	4640A	71	86	3.1	0.58
	Controls (*n*=9)	–	99±22	102±22	2.3±0.4	0.77±0.11
Succinate	Patient E	3635A/8551C	90	123	2.2	–
	Patient M	4640A	122	186	2.0	–
	Controls (*n*=9)	–	130±37	161±50	1.5±0.1	–

amino acid 110 (Table 3). The 8551C mutation substitutes a leucine for a highly conserved phenylalanine at ATP6 amino acid 9 and also alters the nucleotide sequence of the overlapping ATP8 gene, although the base change is silent in the ATP8 reading frame. Mutation-specific restriction enzyme assays for these variants revealed that these mutations were homoplasmic in the proband mtDNA but were not present in 20 haplogroup J-matched controls. Further, these mutations have not been previously reported in the literature, nor are they recorded in the human mtDNA database, MITOMAP (http://www.gen.emory.edu/mitomap.html; Kogelnik et al. 1997).

The mtDNA from the proband of Family M harbored 30 base changes relative to the Cambridge reference sequence, 23 of which were known polymorphisms. Of the remaining seven mutations, six were silent variants and one, a C to A transversion at np 4640 of the ND2 gene, represented a novel missense mutation. The 4640A mutation replaces a poorly conserved isoleucine with a methionine at ND2 amino acid 57. Mutation-specific restriction enzyme analysis revealed that the mutation was homoplasmic. Polymorphisms at nps 11467, 12308, 12372, and 16343 identified the proband mtDNA as European haplogroup U3 (Macaulay et al. 1999). The 4640A mutation has not been previously reported in the literature, is not recorded in MITOMAP, and was not found in 65 random European controls (data not shown).

Functional analysis of OXPHOS

To determine whether the mitochondria of Families E (3635A/8551C mutations) and M (4640A mutation) exhibited OXPHOS defects, we performed respiration and enzymological studies on proband lymphoblast and transmitochondrial cybrid mitochondria (Tables 4, 5). Lymphoblast ADP-stimulated (state III) oxygen consumption rates with complex I substrates (malate + pyruvate or glutamate) were reduced by approximately 55% for Family E and 25% for Family M. Similar reductions were found for the succinate-normalized state III ratios. Uncoupled respiration rates were also lowered by roughly 60% and 35% for Families E and M, respectively. Mutant ADP/O ratios were equivalent to control ratios. All respiration rates and ADP/O ratios with succinate (complex II) as the substrate were essentially identical to controls.

The state III rate, uncoupled rate, and state III ratio reductions found in lymphoblasts were also apparent in WAL-2A-ρ°-derived transmitochondrial cybrids, although the magnitude of the state III ratio reduction for the 3635A/8551C cybrid was not as dramatic as that found in lymphoblasts. ADP/O ratios were not reduced in the LHON patient cybrids. State III and uncoupled rates and ADP/O ratios with succinate as a substrate were similar to those of controls.

Analysis of OXPHOS enzyme-specific activity in lymphoblast mitochondria from Family E or Family M did not suggest a complex I defect in submitochondrial particles. This was true for absolute and citrate-synthase-nor-

Table 5 Specific enzyme activity of Siberian LHON patient and control lymphoblasts and cybrids (*CS* citrate synthase)

Individual	Mutation	Mean specific activity (nmol of substrate/min per mg mitochondrial protein)		
		Complex I	CS	Complex I/CS ratio
Lymphoblasts				
Patient E	3635A/8551C	55	338	0.16
Patient M	4640A	91	837	0.11
Controls (*n*=22)	–	53±20	599±177	0.08±0.02
Cybrids				
Patient E	3635A/8551C	46	576	0.08
Patient M	4640A	64	645	0.10
Controls (*n*=9)	–	44±14	471±176	0.10±0.03

malized complex I activity. The same result was found in cybrid mitochondria. Thus, these novel mutations impair oxygen utilization in intact mitochondria but do not impart a complex I enzymological defect.

Discussion

The extensive genetic and biochemical characterization of patient mitochondria from five families exhibiting maternal transmission of optic atrophy has demonstrated the presence of LHON in Russia. Three of our families harbor a known primary LHON mutation. The 11778A mutation is the most common LHON mtDNA mutation worldwide (found in roughly 50% of all reported LHON cases) and has been detected in two (Families D and I) of the Siberian families. Family I also contains the 4216C and 13708A mutations, indicating that it belongs to European mtDNA haplogroup J, which accounts for roughly 9% of European-derived mtDNAs (Torroni et al. 1996). In North American and European LHON families, the 11778A mutation is associated with haplogroup J three to six times more frequently than expected, suggesting that expression of this variant is enhanced by haplogroup J polymorphism(s) (Brown et al. 1995, 1997; Torroni et al. 1997). Interestingly, Family I had more affected females than affected males, which is atypical for LHON. The 3460A mutation, which accounts for approximately 15% of all Caucasian LHON patients, was found in one (Family S) Siberian LHON family.

Family E: 3635A and 8551C mutations

Two Siberian families harbored novel complex I mutations. The 3635A ND1 and 8551C ATP6 missense mutations were found in proband mtDNA from Family E, which was characterized by maternal transmission of optic atrophy through four generations. In this family, six of the eight affected individuals were male, consistent with other LHON mutations. Both mutations are homoplasmic, alter highly evolutionarily conserved amino acids, and are not found in haplotype-matched controls, nor have they been reported in MITOMAP.

A role for the ND1 3635A mutation in the etiology of LHON was confirmed by demonstrating a strong complex-I-linked respiration defect, which was linked to the mtDNA by cybrid transfer. However, this mutation did not cause a complex I enzyme defect. The complex V 8551C mutation was probably not pathogenic, since it did not alter ADP/O ratios, or reduce complex-I- *and* complex-II-linked (succinate) respiration rates. It remains possible, however, that this novel variant is mildly pathogenic.

Overall, it is likely that the 3635A mutation contributes to LHON in Family E as it is a unique complex I missense mutation that is not found in haplotype-matched controls and is associated with a complex-I-linked respiration defect in lymphoblasts and cybrids. Further, the mutation alters a phylogenetically conserved amino acid located in the middle of one (span C) of eight proposed hydrophobic and membrane-spanning segments of the ND1 polypeptide (Fearnley and Walker 1992). Finally, the 4216C and 13708A LHON secondary mutations found in Family E may synergistically exacerbate the expression of the 3635A mutation, as postulated for the haplogroup-J-associated 11778A and 14484C primary LHON mutations (Brown et al. 1995, 1997; Torroni et al. 1997).

Family M: 4640A mutation

It is possible that the 4640A ND2 mutation contributes to the LHON of Family M. The 4640A mutation is a unique, complex I missense mutation in a family exhibiting maternal transmission of optic atrophy, primarily in males. The mutation is homoplasmic and has not been reported in the literature or MITOMAP (Kogelnik et al. 1997). It alters a weakly conserved amino acid residue that is situated immediately upstream from a postulated membrane spanning α-helix in the ND2 polypeptide (Fearnley and Walker 1992). The 4640A variant was associated with a respiration defect by using NADH-dehydrogenase-linked substrates, which transferred with the mtDNA in cybrids.

Conclusions

These data demonstrate that the spectrum of Siberian LHON mtDNA mutation is similar to that of North America and Europe, although novel mutations clearly occur.

The pattern of OXPHOS dysfunction (significant respiration defect in the absence of strong enzymological defect) associated with the 3635A and 4640A mutations is similar to the functional defects found in 11778A mutant mitochondria. It has been postulated that the 11778A mutation impairs the proton translocation function of complex I and/or destabilizes the ubisemiquinone intermediates of the enzyme (Degli Esposti et al. 1994; Brown 1999). Although these two mutations have only been found in single LHON families, the available data suggests that they cause LHON. Hence, LHON appears to be primarily caused by the known "common" mtDNA mutations in most populations, although a small percentage of cases will result from novel mutations. These new mutations will continue to provide us with new insights into the pathophysiology of mitochondrial disease.

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