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Mitochondrial DNA mutation m.3635G>A may be associated with Leber hereditary optic neuropathy in Chinese

A-Mei Zhang [a,c], Yang Zou [a,c], Xiangming Guo [b], Xiaoyun Jia [b], Qingjiong Zhang [b,*], Yong-Gang Yao [a,*]

[a] Key Laboratory of Animal Models and Human Disease Mechanisms of Chinese Academy of Sciences & Yunnan Province, Kunming Institute of Zoology, Kunming, Yunnan 650223, China
[b] State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangzhou 510060, China
[c] Graduate School of the Chinese Academy of Sciences, Beijing 100039, China

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ABSTRACT

Leber hereditary optic neuropathy (LHON) was the first disease to be linked to the presence of a mitochondrial DNA (mtDNA) mutation. Nowadays over 95% of LHON cases are known to be caused by one of three primary mutations (m.11778G>A, m.14484T>C, and m.3460G>A). Reports for other (rare) primary mutations in LHON patients are not infrequent. Among those is the mutation m.3635G>A in the *MT-ND1* gene which was reported to be pathogenic in a Russian LHON family. In this study, we report on a Chinese family with clinical features of LHON but without any of the three well-known primary mutations. Analysis of the complete mitochondrial genome in the proband revealed the presence of m.3635G>A and m.6228C>T, along with a full array of other variants that suggest the haplogroup M7b1. Evolutionary analysis indicates that site 3635, but not 6228, is highly conserved in vertebrates. Protein secondary-structure modeling for the MT-ND1 protein harboring amino acid change S110N indicates that mutant m.3635G>A decreases the protein hydrophobicity. Our current observations provide further support for a pathogenic role of m.3635G>A in patients with LHON.

Leber hereditary optic neuropathy (LHON; MIM 535000) is one of the most common mitochondrial disorders. In over 95% of LHON cases the disease is caused by the presence of one of three primary mutations (m.11778G>A in the *MT-ND4* gene, m.3460G>A in the *MT-ND1* gene, and m.14484T>C in the *MT-ND6* gene) [1–4]. All three of these mutations impair the function of the respiration chain complex I, but the pathogenesis of LHON has not been well understood [1–4]. Incomplete penetrance and gender bias of LHON indicates that there must be other factors associated with LHON, such as nuclear gene(s), mtDNA background, and environmental factors [1–3,5,6]. The overall primary mutation spectra of east Eurasian and west Eurasian populations are different, and mutation m.11778G>A is more common in LHON patients from China than from Europe [5,7]. Moreover, the mtDNA haplogroups/backgrounds that affect the clinical expression of LHON are also different in Chinese and western European populations. We recently found that haplogroup M7b1'2 increases the risk of LHON, whereas haplogroup M8a has a protective effect for the penetrance of LHON in Chinese with m.11778G>A [8].

Reports for other (rare) primary mutations in LHON patients are not infrequent (cf. http://www.mitomap.org/rimtab1.html). However, many of these mutations were only found in single LHON families or singleton cases. There is a lack of independent observation, not to mention functional verification. Therefore, the claimed pathogenicity of many of those mutations awaits further confirmation. Mutation m.3635G>A in the *MT-ND1* gene was first reported to be pathogenic in a Russian LHON family [9]. This mutation changes the serine at the 110th amino acid position in the MT-ND1 protein to asparagine and causes a respiration defect with complex-I-linked substrates, although the specific activity of complex I in patient's lymphoblasts and transmitochondrial cybrids is not reduced [9]. Since the initial characterization of m.3635G>A in the Russian LHON family, there have not been any studies to support the presence of this mutation as a cause of LHON. In a survey looking at the mtDNA mutation spectra in Chinese patients with LHON or suspected LHON, we have identified m.3635G>A in one Chinese LHON family. This observation provides further support for a pathogenic role of m.3635G>A in patients with LHON.

* Corresponding authors. Address: Key Laboratory of Animal Models and Human Disease Mechanisms, Kunming Institute of Zoology, Chinese Academy of Sciences, Jiaochang Donglu 32, Kunming, Yunnan 650223, China. Fax: +86 871 5180085 (Y.-G. Yao), +86 20 87333271 (Q. Zhang).
E-mail addresses: qingjiongzhang@yahoo.com (Q. Zhang), ygyaozh@gmail.com, ygyaozh@yahoo.com (Y.-G. Yao).

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Materials and methods

Patient. We recently launched a comprehensive survey for mtDNA mutations in Chinese patients with LHON or suspected LHON [7,8,10–12] and have collected samples from over 1500 patients/families. Among them, was a LHON family (Le1143) from Guangdong Province, China, which was seen at the Pediatric and Genetic Clinic of the Eye Hospital, Zhongshan Ophthalmic Center. Informed consents conforming to the tenets of the Declaration of Helsinki and following the guidance of sample collection of Human Genetic Disease (863 program) by the Ministry of Public Health of China were obtained from each participant prior to the study. The institutional review boards of the Zhongshan Ophthalmic Center and the Kunming Institute of Zoology approved this study.

mtDNA genome sequencing. Total genomic DNA was isolated from blood using a standard phenol/chloroform method. The complete mtDNA genome of the proband (III:4) was amplified and sequenced using a modified method as described in our previous study [11].

Data analyses. Sequences were handled by the DNAstar program (DNAS Inc., Madison, WI, USA). Sequence variation was scored relative to the revised Cambridge Reference Sequence (rCRS) [13]. We followed the East Asian mtDNA phylogenetic tree [14] to classify the LHON mtDNA. We defined the uniqueness of the mtDNA variants in this family by an exhaustive database search following the available guidelines [15]. Two other LHON mtDNA sequences, one from family E in Brown et al. [9] showing mutation m.3635G>A [GenBank Accession No. EU807741] and one from family Le924 containing variant m.6228C>T in our recent study [8] [FJ198224], were considered for comparison. Sequence variations in the mtDNA of the proband and the two other sequences are presented together in a mtDNA tree. Evolutionary conservation analysis for certain mtDNA variants was performed using the same approach as in our previous study [11]. We performed secondary-structure modeling for the MT-ND1 protein harboring the mutation m.3635G>A and compared the results to the wild-type (rCRS) by using the TMpred program (http://www.ch.embnet.org/software/TMPRED_form.html). This allowed us to analyze the potential change of hydrophobicity between the mutant and wild-type MT-ND1 protein.

Results

Clinical features

The proband is a 18-year-old male. When he first visited the clinic he had been suffering from severely reduced visual acuity of 3–4 months duration without accompanying other symptoms. He had good visual acuity before but at the time of his attendance the visual acuity was 0.04 for both eyes. Ocular examination revealed temporal optic atrophy. His grandmother-in-law had

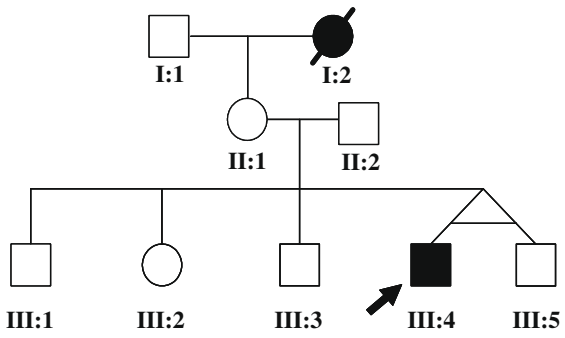

Fig. 1. Pedigree information for a Chinese LHON family (Le1143) with m.3635G>A. Affected individuals are marked by filled symbols. The proband, marked with an arrow, provided the sample for complete mtDNA sequencing.

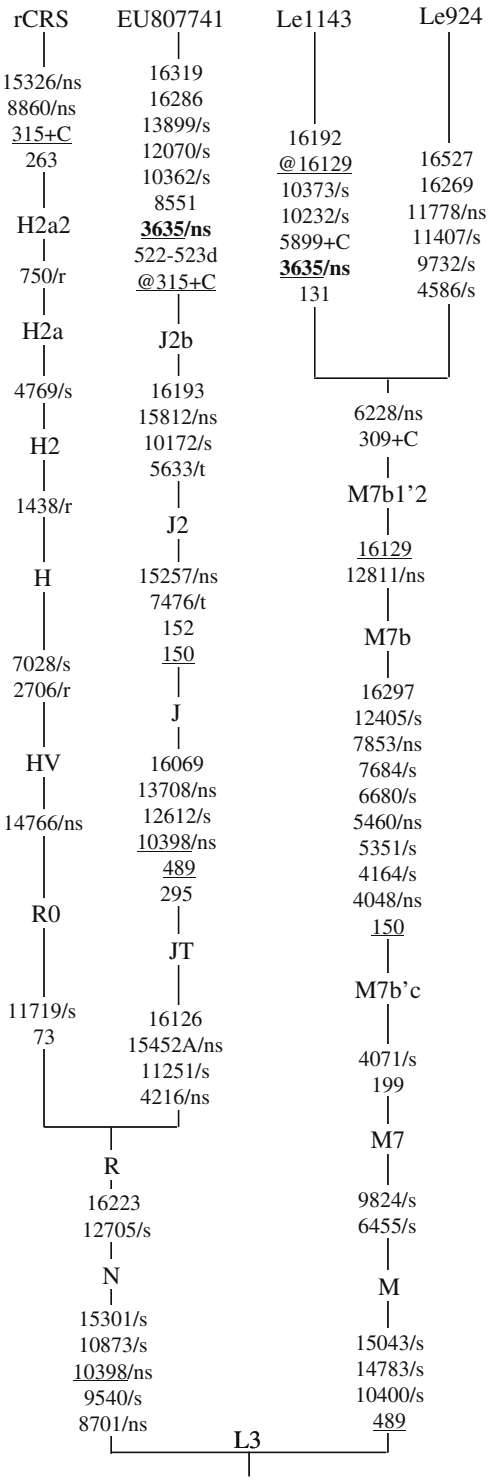

Fig. 2. Classification tree of the proband's mtDNA (family Le1143), the two other reported mtDNAs, and the revised Cambridge reference sequence (rCRS) [13]. The mtDNA sequences EU807741 and FJ198224 (family Le924) are taken from Brown et al. [9] and our previous study [8], respectively. The order of mutations on each uninterrupted branch section is arbitrary. Recurrent mutations are underlined and back mutations are marked by "@". The synonymous and non-synonymous coding-region variants in the mtDNA sequences are denoted by "/s" and "/ns", respectively. Variations in the ribosomal RNA genes and tRNA genes are denoted by "/r" and "/t", respectively.

suffered a similar onset of severely reduced visual acuity (Fig. 1). The penetrance of LHON in family Le1143 is relatively low (16.7%) when compared to most families that we have described before [7,8,11,12].

mtDNA sequence variations and evolutionary analysis

Analysis of the proband's complete mtDNA genome showed the presence of 46 homoplasmic variations compared to the rCRS and suggest he is a member of haplogroup M7b1 (Fig. 2). This haplogroup is common in Chinese and has a higher frequency in South China than in other regions across China [16]. We recently found that mtDNA background M7b1'2 (M7b1 is a subhaplogroup of this haplogroup) increased the penetrance of LHON in patients with the mutation m.11778G>A [8]. The other private nucleotide substitutions in the proband's family are m.131T>C in the control region, m.3635G>A in the *MT-ND1* gene, m.5899insC in the short non-coding region between $tRNA^{tyr}$ (*MT-TY*) and cytochrome *c* oxidase I (*MT-COI*) genes, m.6228C>T in the *MT-COI* gene, and m.10232A>G and m.10373G>A in the *MT-ND3* gene. None of these variations are novel based on the web-search [15]. Among them, m.3635G>A and m.6228C>T are non-synonymous. Evolutionary analysis based on 10 vertebrate species shows that m.3635G>A changes a highly conserved serine to asparagine at position 110 in MT-ND1. Whereas m.6228C>T changes leucine to phenylalanine at position 109 in MT-COI and this site is not conserved in the human (Fig. 3). The complete mtDNA sequence of the proband has been deposited in GenBank under accession number GQ202273.

Analysis of MT-ND1 protein secondary-structure

As shown in Fig. 4, the amino acid change S110N caused by m.3635G>A decreased the hydrophobicity of the MT-ND1 protein, despite the fact that both amino acids are hydrophilic. In contrast, the known primary mutation m.3460G>A affected the hydrophilicity of a proximal domain. This result suggested that the stability of the oxidative phosphorylation (OXPHOS) supercomplexes may alter in the presence of m.3635G>A, thus leading to a potential pathogenicity [17]. The L109F change caused by variant m.6228C>T in MT-COI did not change the hydrophobicity of the MT-COI protein (data not shown).

Discussion

Although, nearly two decades have elapsed since the first identification of the effect of a mtDNA mutation in LHON [18], the exact pathogenesis of this disease has not been well elucidated [1–4].

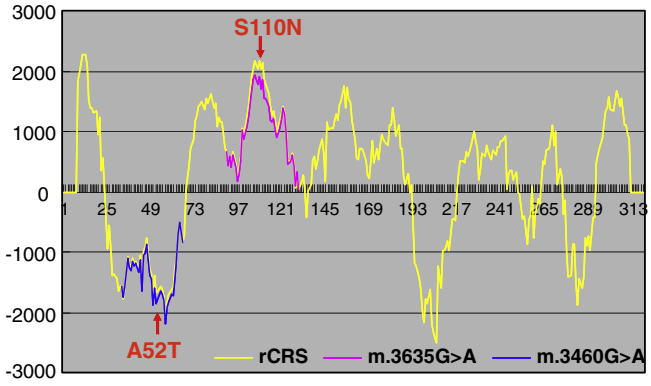

Fig. 4. A hydrophobicity chart for the MT-ND1 protein predicted by the TMpred program. The hydrophobicity of the MT-ND1 proteins harboring the specific amino acid change caused by m.3635G>A and m.3460G>A are compared to the wild-type protein (rCRS).

The etiology of the disease in many patients with the clinical features of LHON remains unclear. In an initial survey looking for mtDNA mutations in LHON patients, we found that the overall distribution patterns of the three primary mutations (m.3460G>A, m.11778G>A, and m.14484T>C) in the Chinese population were significantly different from those found in western European populations [5,7]. We also identified a co-existence of the LHON primary mutation m.11778G>A and a deafness-associated mutation m.1555A>G in a family with a high penetrance of LHON [12]. Moreover, we demonstrated that mtDNA haplogroups M7b1'2 and M8a had quite different roles on the clinical expression of LHON in Chinese [8]. All these studies undoubtedly broadened our knowledge about LHON expression in Chinese. Because the majority of patients with similar LHON features do not have the three known primary mutations, identifying new mtDNA mutation(s) associated with LHON will have important implications for clinical diagnosis and genetic counseling.

In this study, we report the identification of a Chinese LHON family in which the proband has the mtDNA mutation m.3635G>A. This mutation, first reported by Brown et al. [9] in a Russian LHON family, could substantially decrease the ADP-stimulated oxygen consumption and succinate-normalized state III ratios in lymphoblast and transmitochondrial cybrid mitochondria from the proband as compared to controls. Mutation m.3635G>A received a pathogenicity score of nine (maximum score of 40) in the complex I pathogenicity scoring system proposed by Mitchell et al. [19]. We searched for mutation m.3635G>A in the Phylotree.org website (http://www.phylotree.org/), where 5794 complete, or near-complete,

Fig. 3. Evolutionary conservation analysis of the amino acid changes for the non-synonymous mtDNA variants m.3635G>A and m.6228C>T identified in family Le1143. The amino acid sequences are compared to that derived from the human reference sequence (*Homo sapiens* (rCRS), GenBank Accession No. J01415) and nine different vertebrate species: gorilla (*Gorilla gorilla* NC_001645), blue whale (*Balaenoptera musculus* NC_001601), mouse (*Mus musculus* AY466499), dog (*Canis familiaris* DQ480502), wolf (*Canis lupus chancos* EU442884), cattle (*Bos taurus* AY526085), horse (*Equus caballus* EF597513), zebrafish (*Danio rerio* NC_002333), and frog (*Rana nigromaculatas* AB043889).

mtDNA sequences were listed (version dated on May 9, 2009), but didn't find any new sequence harboring m.3635G>A. However, a Google search with "G3635A" as described in our recent study [15] identified three further LHON cases with this mutation in Chinese people: two mtDNAs sequences are available from GenBank (Accession Nos. FJ969382 and FJ969383) and belong to haplogroup R11a and D4g, respectively. By using a phylogenetic approach [20–22], we proofread the two complete mtDNA sequences and identified several sequencing errors. The third case, said to be from haplogroup F1, was only mentioned in an abstract indexed at website (http://www.medcon.org.cn/2009/cmao/cn/news.asp?abid=3238.html). Taken together, it is obvious that m.3635G>A occurs independently in Chinese LHON patients from different mtDNA backgrounds. Although, we failed to get more blood samples from family Le1143 to perform further functional assays for m.3635G>A, we believe this mutation is associated with LHON in Chinese families based on the following lines of evidence. First, m.3635G>A has not been found in the available complete mtDNA sequences across the world (most of them are from general populations) but exists in five LHON families without the three primary mutations ($p < 0.01$). Second, protein secondary-structure modeling for the MT-ND1 protein with S110N change showed a decrease of hydrophobicity. Third, this mutation was found to cause a respiration defect with complex-I-linked substrates [9].

Haplogroups M7b1'2 and J have been found to increase penetrance in East Asian and western European LHON families with m.11778G>A, respectively [5,8]. Coincidentally, the proband in this study and the family reported by Brown et al. [9] belonged to these two high-risk haplogroups. Whether the two mtDNA haplogroups affect the penetrance of LHON in patients with m.3635G>A remains unresolved.

Intriguingly, m.6228C>T in family Le1143 was also present in LHON family (Le924) with m.11778G>A from our previous studies [8]. Both families, Le924 and Le1143, belong to haplogroup M7b1'2 but have different penetrance rates (33.3% versus 16.7%). Because the amino acid change L109F caused by m.6228C>T in MT-COI is not conserved in the human and does not affect the hydrophobicity of this protein, we are inclined to believe that m.6228C>T might not be pathogenic. It is possible that different primary mutations in the two families may contribute to the variance of LHON penetrance. Additional evidence will be needed to test this possibility.

In summary, we identified mutation m.3635G>A in a Chinese LHON family without the three primary mutations. This mutation has been identified in three other Chinese LHON families based on information from web-searches and in one Russian LHON family [9]. Further study will be essential to verify the pathogenic role of mutation m.3635G>A in LHON.

Acknowledgments

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References

[1] V. Carelli, F.N. Ross-Cisneros, A.A. Sadun, Mitochondrial dysfunction as a cause of optic neuropathies, Prog. Retin. Eye Res. 23 (2004) 53–89.
[2] P.Y. Man, D.M. Turnbull, P.F. Chinnery, Leber hereditary optic neuropathy, J. Med. Genet. 39 (2002) 162–169.
[3] M.-Y. Yen, A.-G. Wang, Y.-H. Wei, Leber's hereditary optic neuropathy: a multifactorial disease, Prog. Retin. Eye Res. 25 (2006) 381–396.
[4] P. Yu-Wai-Man, P.G. Griffiths, G. Hudson, P.F. Chinnery, Inherited mitochondrial optic neuropathies, J. Med. Genet. 46 (2009) 145–158.
[5] G. Hudson, V. Carelli, L. Spruijt, M. Gerards, C. Mowbray, A. Achilli, A. Pyle, J. Elson, N. Howell, C. La Morgia, M.L. Valentino, K. Huoponen, M.-L. Savontaus, E. Nikoskelainen, A.A. Sadun, S.R. Salomao, R. Belfort Jr., P. Griffiths, P.Y. Man, R.F. de Coo, R. Horvath, M. Zeviani, H.J. Smeets, A. Torroni, P.F. Chinnery, Clinical expression of Leber hereditary optic neuropathy is affected by the mitochondrial DNA-haplogroup background, Am. J. Hum. Genet. 81 (2007) 228–233.
[6] G. Hudson, V. Carelli, R. Horvath, M. Zeviani, H.J. Smeets, P.F. Chinnery, X-Inactivation patterns in females harboring mtDNA mutations that cause Leber hereditary optic neuropathy, Mol. Vis. 13 (2007) 2339–2343.
[7] X. Jia, S. Li, X. Xiao, X. Guo, Q. Zhang, Molecular epidemiology of mtDNA mutations in 903 Chinese families suspected with Leber hereditary optic neuropathy, J. Hum. Genet. 51 (2006) 851–856.
[8] Y. Ji, A.-M. Zhang, X. Jia, Y.-P. Zhang, X. Xiao, S. Li, X. Guo, H.-J. Bandelt, Q. Zhang, Y.-G. Yao, Mitochondrial DNA haplogroups M7b1'2 and M8a affect clinical expression of Leber hereditary optic neuropathy in Chinese families with the m.11778G –>A mutation, Am. J. Hum. Genet. 83 (2008) 760–768.
[9] M.D. Brown, S. Zhadanov, J.C. Allen, S. Hosseini, N.J. Newman, V.V. Atamonov, I.E. Mikhailovskaya, R.I. Sukernik, D.C. Wallace, Novel mtDNA mutations and oxidative phosphorylation dysfunction in Russian LHON families, Hum. Genet. 109 (2001) 33–39.
[10] Y. Ji, X. Jia, Q. Zhang, Y.-G. Yao, MtDNA haplogroup distribution in Chinese patients with Leber's hereditary optic neuropathy and G11778A mutation, Biochem. Biophys. Res. Commun. 364 (2007) 238–242.
[11] H.-W. Wang, X. Jia, Y. Ji, Q.-P. Kong, Q. Zhang, Y.-G. Yao, Y.-P. Zhang, Strikingly different penetrance of LHON in two Chinese families with primary mutation G11778A is independent of mtDNA haplogroup background and secondary mutation G13708A, Mutat. Res. 643 (2008) 48–53.
[12] A.-M. Zhang, X. Jia, Y.-G. Yao, Q. Zhang, Co-occurrence of A1555G and G11778A in a Chinese family with high penetrance of Leber's hereditary optic neuropathy, Biochem. Biophys. Res. Commun. 376 (2008) 221–224.
[13] R.M. Andrews, I. Kubacka, P.F. Chinnery, R.N. Lightowlers, D.M. Turnbull, N. Howell, Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA, Nat. Genet. 23 (1999) 147.
[14] Q.-P. Kong, H.-J. Bandelt, C. Sun, Y.-G. Yao, A. Salas, A. Achilli, C.-Y. Wang, L. Zhong, C.-L. Zhu, S.-F. Wu, A. Torroni, Y.-P. Zhang, Updating the East Asian mtDNA phylogeny: a prerequisite for the identification of pathogenic mutations, Hum. Mol. Genet. 15 (2006) 2076–2086.
[15] H.-J. Bandelt, A. Salas, R.W. Taylor, Y.-G. Yao, Exaggerated status of "novel' and "pathogenic" mtDNA sequence variants due to inadequate database searches, Hum. Mutat. 30 (2009) 191–196.
[16] Y.-G. Yao, Q.-P. Kong, H.-J. Bandelt, T. Kivisild, Y.-P. Zhang, Phylogeographic differentiation of mitochondrial DNA in Han Chinese, Am. J. Hum. Genet. 70 (2002) 635–651.
[17] R. Pello, M.A. Martin, V. Carelli, L.G. Nijtmans, A. Achilli, M. Pala, A. Torroni, A. Gómez-Durán, E. Ruiz-Pesini, A. Martinuzzi, J.A. Smeitink, J. Arenas, C. Ugalde, Mitochondrial DNA background modulates the assembly kinetics of OXPHOS complexes in a cellular model of mitochondrial disease, Hum. Mol. Genet. 17 (2008) 4001–4011.
[18] D.C. Wallace, G. Singh, M. Lott, J. Hodge, T. Schurr, A. Lezza, L. Elsas, E. Nikoskelainen, Mitochondrial DNA mutation associated with Leber's hereditary optic neuropathy, Science 242 (1988) 1427–1430.
[19] A.L. Mitchell, J.L. Elson, N. Howell, R.W. Taylor, D.M. Turnbull, Sequence variation in mitochondrial complex I genes: mutation or polymorphism?, J Med. Genet. 43 (2006) 175–179.
[20] Y.-G. Yao, A. Salas, C.M. Bravi, H.-J. Bandelt, A reappraisal of complete mtDNA variation in East Asian families with hearing impairment, Hum. Genet. 119 (2006) 505–515.
[21] H.-J. Bandelt, Y.-G. Yao, C.M. Bravi, A. Salas, T. Kivisild, Median network analysis of defectively sequenced entire mitochondrial genomes from early and contemporary disease studies, J. Hum. Genet. 54 (2009) 174–181.
[22] A. Salas, Y.-G. Yao, V. Macaulay, A. Vega, Á. Carracedo, H.-J. Bandelt, A critical reassessment of the role of mitochondria in tumorigenesis, PLoS Med. 2 (2005) e296.