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Mitochondrial DNA haplogroup distribution in Chaoshanese with and without myopia

Qin Wang,[1] Panfeng Wang,[1] Shiqiang Li,[1] Xueshan Xiao,[1] Xiaoyun Jia,[1] Xiangming Guo,[1] Qing-Peng Kong,[2] Yong-Gang Yao,[3] Qingjiong Zhang[1]

[1]State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangzhou, China; [2]State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, China; [3]Key Laboratory of Animal Models and Human Disease Mechanisms of Chinese Academy of Sciences & Yunnan Province, Kunming Institute of Zoology, Kunming, China

Purpose: Mitochondrial DNA (mtDNA) haplogroups affect the clinical expression of Leber hereditary optic neuropathy, age-related macular degeneration, and other diseases. The objective of this study is to investigate whether an mtDNA background is associated with myopia.

Methods: Blood DNA was obtained from 192 college students, including 96 individuals with moderate-to-high myopia and 96 controls without myopia. All the subjects were from a well-known isolated population living in the Chaoshan area of east Guangdong Province and speaking one of the four major dialects in southern China. The mtDNA haplogroups in the 192 subjects were determined by sequencing the mtDNA control region and partial coding regions as well as by analysis of restriction fragment length polymorphisms. Each mtDNA was classified according to the updated version of the Eastern Asian haplogroup system.

Results: Sixteen mtDNA haplogroups were recognized in the 192 subjects. The overall matrilineal structures of the samples with and without myopia were similar and had genetic imprints showing their ethno-origin. There was no statistical difference in frequencies of haplogroup distribution between subjects with and without myopia (χ^2 test, p=0.556).

Conclusions: We failed to identify clues that suggest an involvement of mtDNA background in the predisposition to myopia.

Mitochondrial bioenergetics is linked to oxidative stress that is associated with aging and neurodegeneration [1-3]. Mitochondria are involved in the production and clearance of reactive oxygen species (ROS), and mutations of mitochondrial DNA (mtDNA) may result in energy deficiency and an increase in oxygen radicals. mtDNA haplogroups, which are determined by a series of characteristic variations and were formed during the origin and migration of modern humans, have been shown to play active roles in several neurodegenerative diseases, including Alzheimer disease [4,5], Parkinson disease [6], and multiple sclerosis [7], despite some of the original claims not being repeated in subsequent studies [8]. In the eye, mtDNA haplogroups have been reported to affect the clinical expression of Leber hereditary optic neuropathy (LHON) in European [9] and Chinese families [10], age-related macular degeneration [11,12], and optic neuritis [13]. The mtDNA haplogroup effect is ethnic specific, as demonstrated in LHON where the haplogroups associated with LHON expression in Chinese populations are different from those in Caucasian populations [10].

Myopia can be caused by excessive reading and close work, which is potentially related to oxidative stress [14-16]. Individuals exposed to hyperbaric oxygen showed a refractive change to myopia [17-19]. On the other hand, high myopia is frequently associated with retinal neurodegeneration [20,21]. Under a similar environment and with similar reading behavior, some individuals develop myopia but others do not, suggesting a genetic background involvement. Linkage and association studies on the nuclear genome have demonstrated the importance of genetic factors in the development of myopia, especially high-grade myopia [22-25]. However, the exact molecular basis for most myopia remains unknown. There have been no reports on the potential association of myopia with the mitochondrial genome, although mtDNA variations and haplogroups are known to be associated with neurodegeneration and oxidative stress.

Chaoshanese is an intriguing, isolated, Han Chinese population that is located in the Chaoshan area, east Guangdong Province. This population has unique features in dialects, life styles, customs, habits, and a population census of 12 million. The Chaoshanese are suggested to be descendents of northern Chinese who immigrated during the Ming Dynasty (1368–1628 A.D.) or earlier [26]. In this study, we analyzed the mtDNA haplogroup distribution frequencies

Correspondence to: Qingjiong Zhang, M.D., Ph.D., Ophthalmic Genetics & Molecular Biology, Zhongshan Ophthalmic Center, Sun Yat-sen University, 54 Xianlie Road, Guangzhou 510060, China; Phone: (+86)-20-87330422; FAX: (+86)-20-87333271; email: qingjiongzhang@yahoo.com

Table 1. Information of the subjects with and without myopia.

	Myopias (M group; n=96)		Controls (NC group; n=96)	
Characteristics	**OD**	**OS**	**OD**	**OS**
Age, mean(SD), y	21.8 (1.3)		21.7 (1.3)	
Females, No. (%)	33 (34.4)		33 (34.4)	
SE, mean (SD), D	−6.52 (1.31)	−6.37 (1.36)	0.27 (0.51)	0.33 (0.44)
AL, mean(SD), mm	26.28 (0.96)	25.22 (1.02)	23.78 (0.72)	23.72 (0.68)
K, mean (SD), D	43.72 (1.42)	43.69 (1.43)	42.75 (1.45)	42.75 (1.46)
ACD, mean (SD), mm	3.78 (0.29)	3.79 (0.31)	3.44 (0.23)	3.47 (0.23)
Partial correlation with SE, r (p value)				
AL	−0.68 (<0.001)	−0.71 (<0.001)	−0.30 (0.003)	−0.34 (0.001)
K	−0.55 (<0.001)	−0.57 (<0.001)	−0.23 (0.025)	−0.21 (0.046)
ACD	0.28 (0.007)	0.19 (0.062)	0.01 (0.939)	0.08 (0.475)

Abbreviations: ACD, anterior chamber depth; AL, axial length; K,corneal curvature; SE, spherical equivalent.

in Chaoshanese with and without myopia to detect the potential association between the mtDNA background and myopia.

METHODS

Subjects: College students were recruited from 12 universities in Guangzhou, China, as part of a project to identify the genetic causes of complex high myopia. In total 2,699 students were examined, including 1,276 individuals with moderate-to-high myopia (spherical refraction at each meridian ≤–4.00D) and 1,423 control individuals without a significant refractive error (with best unaided visual acuity of 1.0 or better and bilateral refraction of a spherical equivalent between −0.50D and +2.00D). For this study, 96 cases (66 males and 33 females, age from 19 to 25) and 96 controls (66 males and 33 females, age from 19 to 26) from the Chaoshan area were selected based on similarities in age, gender, educational background, and ethnic origin (local dialect and places where they grew up). Detailed clinical information on the subjects is listed in Table 1. The 96 cases were selected based on the following criteria: 1) born in the Chaoshan area and can speak the Chaoshanese dialect; 2) best corrected visual acuity of 0.8 or better; 3) spherical refraction at each meridian ≤–4.00D; 4) no other known eye or related systemic diseases; 5) no family history of high myopia; and 6) myopia occurred at age 7 years or older). The 96 controls met the following criteria: 1) born in the Chaoshan area and can speak the Chaoshanese dialect; 2) best unaided visual acuity of 1.0 or better; 3) bilateral refraction between −0.50D and +2.00D (spherical equivalent); 4) no other known eye or related systemic diseases; and 5) no family history of high myopia or hyperopia. Case and control individuals without complete data, especially data for the measurement of IOL Master V5 (Carl Zeiss Meditec AG, Jena, Germany), were excluded.

The refractive error was measured with cycloplegic autorefraction after mydriasis (Mydrin®-P, a tropicamide compound; Santen Pharmaceutical Co., Ltd., Osaka, Japan). Ophthalmologic examinations were performed by ophthalmologists (Q.Z. and X.G.). Blood of each subject was drawn from superficial veins of the arm by using disposable syringe after sterilization of skin. Serum was removed after centrifugation of the blood and the remaining leukocytes were separated from red blood cells by hypotonic hemolysis. Leukocytes were digested by proteinase K. The digested leukocytes were then extracted by using phenol/chloroform solution. The supernatant was mixed with cold alcohol to generate a genomic DNA pellet. Genomic DNA was dissolved in TE buffer. Informed consent conforming to the tenets of the Declaration of Helsinki was obtained from each participant before the study. The Institutional Review Board of Zhongshan Ophthalmic Center approved this study.

Mitochondrial DNA haplogroup classification: mtDNA sequence variations were scored for each sample relative to the revised Cambridge reference sequence [27]. We followed the same strategy and amplification and sequencing methods as described by Yao et al. [28], which have been used and optimized in our recent studies [10,29]. Each mtDNA was categorized according to the methods described by Yao et al. [28] and Kong [30]. Briefly, the first hypervariable segment of the mtDNA control region from 16,001 to 16,497 (HVS-I) was amplified and sequenced for each sample to allow a preliminary classification of the haplogroups. The second hypevariable segment from 30 to 407 (HVS-II) and two coding region segments (regions 2,797-3,273 and 10,171-10,659) were amplified and sequenced in certain samples to justify the haplogroup status based on the preliminary haplogroup status inferred from HVS-I. In addition, all samples were screened for the 9-bp deletion in the COII/$tRNA^{lys}$ region by nondenaturing polyacrylamide gel (8%) electrophoresis to determine the haplogroup B status. Furthermore, haplogroups A, D, and M7 were also genotyped by restriction fragment length polymorphism (RFLP) to

Table 2. mtDNA haplogroup distribution frequencies (%) of subjects with and without myopia.

Haplogroup	Myopias (CS1M, n=96)	Controls (CS1NC, n=96)	CS1** (n=192)	CS2# (n=102)
B	19.79	18.75	19.3	16.7
F	15.63	16.67	16.2	19.6
M7	12.50	10.42	11.5	13.7
R†	2.08	6.25	4.2	1.9
M33	0	1.04	0.5	0.0
D	26.04	22.92	24.5	25.5
M10	3.13	2.08	2.6	2.9
M12	1.04	0	0.5	0.0
A	5.21	2.08	3.6	2.9
G	2.08	4.17	3.1	2.9
M8‡	4.17	9.38	6.8	5.9
N9a	2.08	4.17	3.1	5.9
Y	1.04	0	0.5	0.9
M*	5.21	2.08	3.6	0.9
χ^2 test	χ^2=11.654	p=0.556	χ^2=6.411	p=0.930

The double asterisk indicates subjects with myopia (CS1M) and without myopia (CS1NC) in the present study. The sharp (hash mark) indicates that the Chaoshanese mtDNA data were taken from a recent report [26]. The dagger indicates that R includes R*, R9b, R9c, and R11 and the double dagger indicates that M8 includes M8a, C, and Z.

further confirm the inferred haplogroup status. We followed the strategy for data quality control according to the rules and guidelines described in previous reports [31,32]. This included careful handling to avoid sample contamination, double checking of the sequence reading of HVS-I and HVS-II to avoid base shift or variation missing, cautiously score transition, transversion, deletion, or insertion. For those regions genotyped by RFLP analysis, randomly selected samples were further confirmed by additional sequencing analysis. Final data of mtDNA haplogroups were independently checked by two coauthors.

Statistical analysis: The haplogroup distribution frequencies between the two groups were analyzed by the Pearson χ^2 test. Principal component analysis was conducted to assess the geographic origin of the study subjects based on the mtDNA haplogroup distribution frequencies. Previously reported Han Chinese mtDNA data, including those from Guangdong Province, and our previously published data were used for comparisons ([26,28] and references therein).

RESULTS

The mtDNA sequence variations and haplogroup classifications of all 192 subjects with and without myopia are listed in Appendix 1. All the lineages belonged to haplogroups that are found in Han Chinese and East Asian populations [28]. Most of the samples could be allocated to the smallest haplogroups, with the exception of seven samples with a status of M*, which could not be further classified based on the available information. Haplogroups D, B, F, and M7 were detected in 25 (26.04%), 19 (19.79%), 15 (15.63%), and 12 (12.50%) subjects with myopia, respectively, accounting for 73.96% of the case subjects. Similarly, these four haplogroups were present in 22 (22.92%), 18 (18.75%), 16 (16.67%), and 10 (10.42%) subjects without myopia, respectively, accounting for 68.75% of the control subjects. The haplogroup distributions between these two groups showed no statistical difference (χ^2 test, χ^2=11.654, p=0.556; Table 2).

The 9-bp (CCCCCTCTA) deletion was found in sample NC960, which belongs to haplogroup F2b. The presence of this deletion in haplogroups D4b1b2 and B (including its subhaplogroups) as a haplogroup-specific variant suggests multiple origins of the 9-bp deletion [33]. We found the southern Han prevalent haplogroups (B, F, M7, and R) and northern Han prevalent haplogroups (D, G, M8a, C, and Z) in subjects with and without myopia but again with no statistical difference (χ^2=1.377, p=0.502) in the distribution of these haplogroups between the two groups.

We performed principal component analysis (Figure 1) based on the mtDNA haplogroup frequencies of the Chaoshanese populations with and without myopia and other reported Han Chinese populations ([26,28] and references therein). The first two principal components accounted for 91.8% of the genetic variation. The south-to-north cline of the populations and the heterogeneity of the southern populations were further confirmed by the second principal component (PC). The Chaoshanese population (marked as CS2 in Figure 1) reported by Wang et al. [26] showed a close affinity to the myopia and control populations (CS1M and CS1NC) in this study, which is consistent with the sampling location. This

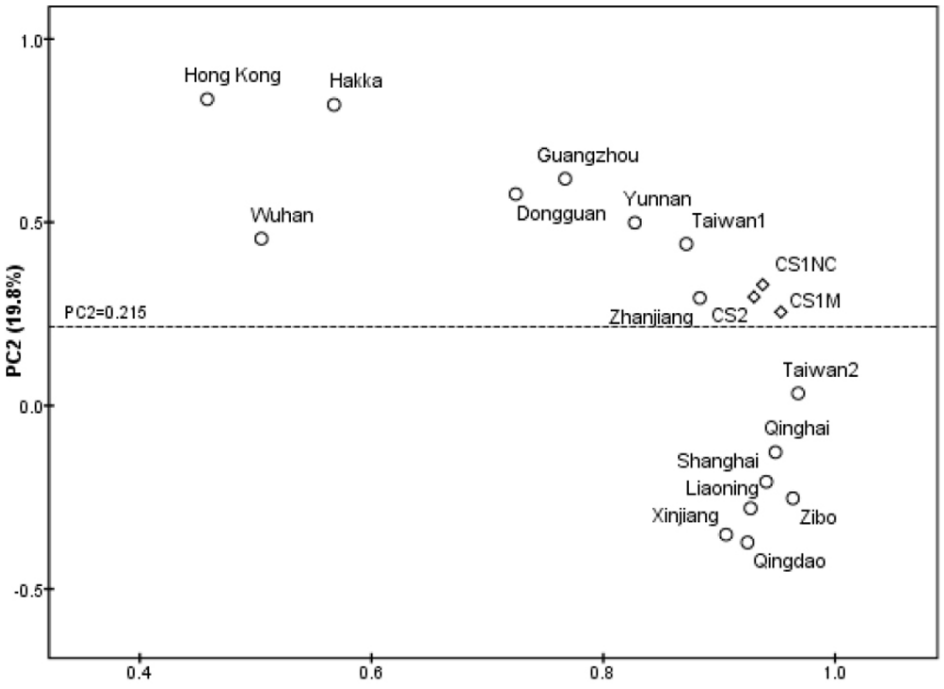

Figure 1. Principal component map of mitochondrial DNA variation. The mitochondrial DNA data (with respect to the haplogroup frequencies in Table 2) of 16 reported regional Han populations were from references ([26, 28] and references therein). The three Chaoshanese populations are marked by diamonds, whereas other Han Chinese populations are labeled by circles with city or province names above the circle. CS1M and CS1NC represent the Chaoshanese populations with (CS1M) and without (CS1NC) myopia in this study. CS2 indicates that the Chaoshanese mtDNA data were taken from a recent report [26]. This figure demonstrated that the Chaoshanese population in this study is identical to the Chaoshanese population previously reported but is different from other Chinese population based on mitochondrial DNA haplogroup analysis.

pattern suggests that the Chaoshanese are relatively homogenous as compared to other Han Chinese.

DISCUSSION

The mitochondrial genome encodes the oxidative phosphorylation system where energy and ROS are generated [1]. Generation of ROS can cause deleterious peroxidation of lipids, modification of proteins, and cleavage of DNA [34], which is referred to as oxidative stress. The retina is particularly sensitive to the deleterious effects of ROS because of its high oxygen consumption and its constant exposure to light [35]. Previous studies have demonstrated that exposure to oxidative stress caused degeneration of photoreceptors and other cells of the neural retina in animal models [36]. The level of lipid peroxidation products may relate to the degree of myopia [37]. Furthermore, single nucleotide polymorphisms in the mitofusin-1 (*MFN1*) and presenilin associated rhomboid-like (*PSARL*) genes are among the clustering peak showing a genetic association with myopia that was mapped to 3q26 (MYP8 locus) [15]. Both *MFN1* and *PSARL* encode mitochondrial membrane proteins that interact with Optic Atrophy 1 (OPA1), a mitochondrial protein known to cause retinal neuron degeneration when mutated [15,38]. Mitochondrial dysfunction, which is caused by mutations in either mtDNA or nuclear-encoded mitochondrial genes, can be a potential target for genetic predisposition to myopia.

In this study, we analyzed mtDNA haplogroup distributional patterns in 192 Chaoshanese individuals (including 96 with myopia and 96 without myopia) to test whether an mtDNA background would affect the clinical expression of myopia. The case and control populations presented a very similar matrilineal structure. We found no statistical difference in the frequency of certain haplogroups between the cases and controls. Principal component analysis demonstrated homogeneity of the Chaoshanese populations analyzed in this study, and this homogeneity had been previously reported [26]. It is unlikely, therefore, that an mtDNA haplogroup would affect myopia. This is in contrast to our recent observation of an increased risk of haplogroup M7b1'2 and a protective role of M8a during the expression of LHON in Chinese families with m.11778G>A [10].

To our knowledge this is the first study to examine the potential association of an mtDNA haplogroup with myopia. We failed to find any evidence that would suggest the involvement of an mtDNA background in the predisposition to myopia. Although the sample size in this study was not large, we have every reason to believe that an mtDNA background is unlikely to play a major role in myopia predisposition as our study has shown that the Chaoshanese population has high genetic homogeneity. This pattern is consistent with the relatively isolated status of the Chaoshanese. The current results may provide guidance for genome-wide association studies of myopia when selecting study populations. The case-control series from the Chaoshan area is a good candidate for such a study.

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Appendix

mtDNA sequence variation and haplogroup classification of 96 subjects with myopia (M) and 96 subjects without myopia (NC). To access the data, click or select the words "Appendix 1." This will initiate the download of a compressed (pdf) archive that contains the file.

The print version of this article was created on 25 February 2010. This reflects all typographical corrections and errata to the article through that date. Details of any changes may be found in the online version of the article.

Appendix 1. mtDNA sequence variation and haplogroup classification of 96 subjects with myopia (M) and 96 subjects without myopia (NC)

SAMPLE[1]	HAPLO-GROUP	SEQUENCE VARIATIONS			RFLP POLYMORPHISMS[3]					
		HVS-I (region 16001-16497) (16000+) [2]	HVS-II (region 30-407)[2]	10171-10659	3010	3206	663e	9820g	5176a	9-bp[4]
M0071	A	223 290 319 362 390					+			2
M161	A	223 290 319 362					+			2
M286	A	223 271 290 319 362					+			2
M960	A	126 223 234 290 319	073 235 263 315+C				+			2
M981	A	086 150 223 290 319 362	073 152 235 263 315+C	10262	G	C	+			2
M347	B	182C 183C 189								1
M1127	B	093 129 183C 189 352 355								1
M904	B	129 183C 189 352 355								1
M0040	B4a	182C 183C 189 217 261 311								1
M767	B4a	051 129 182C 183C 189 217 261 354								1
M1052	B4a	178 182C 183C 189 217 261								1
M632	B4b1	136 183C 189 217 218 239 248	073 263 309+C 315+C							1
M1108	B4c	051 140 182C 183C 189 217 274 335 362								1
M246	B4c	182C 183C 189 214 217 274 311 335								1
M865	B4c	129 140 183C 189 217 274 335								1
M318	B5a	092 140 164 172 182C 183C 189 266G	073 146 210 263 309 315+C							1
M437	B5a	140 183C 189 234 266A								1
M589	B5a	140 182C 183C 189 260 266G								1
M622	B5a	140 182C 183C 189 260 266A 274								1
M744	B5a	140 182C 183C 189 266A 362								1
M1030	B5a	140 183C 189 260 266A 274								1
M967	B5b	140 183C 189 243 355								1
M805	B5b2	111 140 183C 189 234 243 463								1
M920	B5b2	111 140 183C 189 234 243 463								1
M0038	D4	223 287 362			A	C			–	2
M0055	D4	092 223 362			A	C			–	2
M0057	D4	223 242 362			A	C			–	2
M0070	D4	223 362			A	C			–	2
M180	D4	092 223 362			A	C			–	2
M548	D4	092 223 362			A	C			–	2
M679	D4	092 223 362		10398 10400	A	C			–	2
M718	D4	086 223 362		10398 10400	A	C			–	2
M732	D4	092 223 362			A	C			–	2

M848	D4	223 274 311 362		10398 10400	A	C		–	2
M213	D4a	129 223 362			A	T		–	2
M312	D4a	129 223 270 362			A	T		–	2
M896	D4a	129 223 265 270 362			A	T		–	2
M958	D4a	129 223 249 311 362			A	T		–	2
M1253	D4a	129 223 362			A	T		–	2
M657	D4a3	129 223 249 278 311 362			A	T		–	2
M867	D4a3	129 223 249 278 311 362		10398 10400	A	T		–	2
M1210	D4a3	129 223 249 278 311 362		10398 10400	A	T		–	2
M1245	D4b1b2	223 287 319 362			A	C		–	1
M463	D5	182C 183C 189 223 357 362	073 150 263 315+C					–	2
M758	D5	164 172 182C 183C 189 223 259 335 362	073 150 263 309+C 315+C	10397 10398 10400				–	2
M1081	D5	182C 183C 189 223 362						–	2
M1129	D5	182C 183C 189 223 362						–	2
M319	D5a2a'c	092 183C 189 223 266 362	10364 10397 10398 10400	10364 10397 10398 10400				–	2
M842	D5a2a'c	092 182C 183C 189 223 266 362	073 146 150 263 309+C 315+C	10397 10398 10400				–	2
M192	F1a	129 172 304	073 249d 263 309+C 315+C	10310 10609					2
M754	F1a	098 129 172 162 304 311	073 249d 263 315+C	10310 10609					2
M830	F1a	172 304	073 153 199 249d 263 315+C	10310 10609					2
M840	F1a	129 172 304 311 362	073 152 249d 263 309+CC 315+C	10310 10609					2
M1110	F1a	129 162 172 304 399	073 249d 263 309+CC 315+C	10310 10609					2
M1232	F1c	111 266 304 311	073 152 249d 263 309+C 315+C	10310 10454 10609					2
M513	F2	209 248 304	073 249d 263 309+C 315+C	10310 10410 10535 10586					2
M534	F2	304 311	073 200 249d 263 309+C 315+C	10310 10532 10535 10586					2
M1043	F2	189 221 304	073 152 195 249d 263 275 309+CC 315+C	10310 10535 10586					2
M1171	F2	172 183C 189 304 355	073 249d 263 309+CC 315+C	10310 10535 10586					2
M638	F2a2	092A 291 304	073 249d 263 309+C 315+C	10310 10535 10586					2
M781	F2a2	092A 140 291 304	073 199 204 249d 263 315+C	10310 10535 10586					2
M936	F2a2	092A 164 291 304	073 249d 263 309+CC 315+C	10310 10535 10586					2
M438	F3a	260 298 355 362	073 249d 263 309+C 315+C	10310 10320	G	C			2
M0063	F4a	207 304 399	073 146 152 249d 263 281 315+C	10310					2
M254	G2a1d	092 223 278 362	073 152 263 309+C 315+C	10398 10400	G	C			2
M471	G2a1d	223 278 362	073 260 263 315+C	10398 10400	G	C			2
M0075	M10a	223 311		10398 10400 10646					2
M1123	M10a	129 223 311		10398 10400 10646					2
M1220	M10a	129 223 311	073 263 309+C 315+C	10245 10398 10400 10646					2
M881	M12	223 234 287 290 311 362		10398 10400					2
M436	M7	223	073 146 152 199 263 309+C 315+C	10398 10400	G	C	+		2
M135	M7b	129 223 297	073 150 189 199 263 309+C 315+C				+		2

M1248	M7b	129 223 297	073 150 199 263 315+C				+				2
M861	M7b	129 223 297	073 150 199 263 309+C 315+C				+				2
M373	M7b1	129 192 223 297					+				2
M656	M7b1	129 192 223 297					+				2
M892	M7b1	129 192 223 297					+				2
M868	M7c	223 293 295	072 73 146 199 249d 263 309+C				+				2
M1109	M7c	223 293 295	073 146 199 263 309+C 315+C				+				2
M0127	M7c1a	295 319	073 146 199 263 315+C				+				2
M0016	M7c3b	CRS	073 146A 199 263 309+C 315+C				+				2
M736	M7c3b	CRS	073 146A 199 263 315+C				+				2
M524	M8a1	184 223 298 319									2
M863	M8a1	184 223 298 319									2
M962	M8a1	184 223 298 319									2
M1096	N9a	223 257A 261 311	073 150 263 309+C 315+C								2
M937	N9a4	092 145 172 223 245 257A 261	073 152 235 263 315+C 375								2
M761	R11	189 311	073 185 189 207 235 263 309+CCC 315+C								2
M1256	R9b1	093 189 288 304 309 390	073 143 183 263 309+C 315+C								2
M0126	Y1a	126 231 266	073 146 152 217 263 309+C 315+C	10398	G	C					2
M1011	Z	185 223 260 298	073 249d 263 309+C 315+C								2
M542	M*	145 192 223 291 304	073 210 263 309+CC 315+C	10398 10400			−	−	+		2
M788	M*	111 172 183C 189 223 273 362 375	073 185 189 195 234 263 309+C 315+C				−	−	+		2
M323	M*	223 271 311		10398 10400							2
M827	M*	093 223 311 362 381		10268 10398 10400	G	C					2
M1076	M*	189 223 311	073 146 150 152 263 309+C 315+C								2
NC985	A	126 223 235 290 319					+				2
NC1045	A	086 223 290 319 362					+				2
NC76	B	183C 189 352 355									1
NC988	B4	183C 189 217 319									1
NC1070	B4	182C 183C 189 217 223 299									1
NC1354	B4a	182C 183C 189 217 240 261									1
NC1166	B4a	182C 183C 189 217 261									1
NC571	B4a	182C 183C 189 217 261									1
NC584	B4a	182C 183C 189 217 261									1
NC629	B4a	182C 183C 189 217 261									1
NC934	B4a	182C 183C 189 217 261									1
NC647	B4b1	136 182C 183C 189 217 309 354									1
NC906	B4b1	136 182C 183C 189 217									1
NC489	B4c1b	140 182C 183C 189 217 274 316 335									1

NC562	B4c1b	129 140 166 183C 189 217 274 335							1
NC640	B4c1b	129 140 179 182C 183C 189 217 274 311 335							1
NC1223	B4c1b	092 129 140 182C 183C 189 217 274 311 335							1
NC496	B5a	140 183C 189 218 266A							1
NC695	B5a	140 183C 189 266A							1
NC1240	B5b	140 183C 189 243							1
NC524	C	093 129 223 298 327 390	073 195 249d 263 309+C 315+C						2
NC576	C	223 298 327	073 146 249d 263 309+C 315+C						2
NC759	C	223 298 327	073 146 249d 263 309+C 315+C						2
NC419	D4	223 266 362				A	C	–	2
NC553	D4	172 223 362				A	C	–	2
NC951	D4	223 362		10398 10400		A	C	–	2
NC993	D4	111 124 223 362		10310 10398 10400		A	C	–	2
NC1063	D4	223 257 264 311 362		10398 10400		A	C	–	2
NC1076	D4	223 362		10398 10400		A	C	–	2
NC1217	D4	092 223 362				A	C	–	2
NC1313	D4	223 362				A	C	–	2
NC851	D4a	129 223 362				A	T	–	2
NC1061	D4a3	129 223 249 278 311 362		10398 10400		A	T	–	2
NC1222	D4a3	129 223 249 265 278 311 362		10398 10400		A	T	–	2
NC801	D4a3	129 223 249 278 311 362		10398 10400		A	T	–	2
NC1411	D4b1b2	223 287 319 362 380				A	C	–	1
NC303	D5	183C 189 223 357 362	073 150 263 309+C 315+C	10397 10398 10400				–	2
NC659	D5	182C 183C 189 223 256 362						–	2
NC722	D5	183C 189 223 362						–	2
NC828	D5	182C 183C 189 223 362						–	2
NC892	D5	148 182C 183C 189 223 362						–	2
NC930	D5	183C 189 223 357 362						–	2
NC1269	D5	136 189 223 362						–	2
NC949	D5	093 182C 183C 189 223 267 362						–	2
NC976	D5a2a	092 164 172 182C 183C 189 223 266 362	073 150 263 315+C	10364 10397 10398 10400				–	2
NC782	F1	153 183C 189 278 300 304 357	073 249d 263 309+CC 315+C	10310 10609					2
NC556	F1a	172 304	073 200 249d 263 309+C 315+C	10310 10609					2
NC903	F1a	129 172 242 304 311	073 249d 263 309+C 315+C	10310 10609					2
NC721	F1a	172 304	073 249d 263 315+C	10310 10604 10609					2
NC717	F1a1	129 162 172 304 399	073 249d 263 309+CC 315+C	10310 10609					2
NC1003	F1a1	129 162 172 304 399	073 152 249d 263 309+C 315+C	10310 10609					2
NC645	F1a1a	129 108 162 172 304	073 150 249d 263 309+C 315+C	10310 10609					2
NC927	F1b	189 304	073 150 152 195 249d 263 309+C 315+C	10310 10609					2

NC704	F1c	111 129 266 304	073 152 249d 263 309+C 315+C	10310 10454 10704							2
NC1087	F1c	111 129 266 304	073 152 249d 263 309+CC 315+C	10310 10454 10609							2
NC604	F2	092A 291 304	073 249d 263 309+C 315+C	10310 10535 10586							2
NC738	F2	140 291 304	073 199 204 249d 263 315+C	10310 10535 10586							2
NC793	F2	209 304	073 249d 263 309+C 315+C	10310 10410 10535 10586							2
NC1295	F2	291 304	073 249d 263 309+C 315+C	10310 10535 10586							2
NC960	F2a	203 304	073 249d 263 309+C 315+C	10310 10535 10586	A	C					1
NC819	F4a	126 140 207 304 362 399	073 146 249d 263 309+CC 315+C	10310							2
NC936	G2a	172 223 227 272 278 319 362		10398 10400							2
NC995	G2a	223 227 278 362		10398 10400							2
NC1027	G2a	086 104 223 278 362		10398 10400							2
NC1195	G2a	223 227 278 362		10398 10400							2
NC630	M10a	93 129 223 311 327		10398 10400 10646							2
NC926	M10a	129 223 311		10398 10400 10646							2
NC1053	M33c	104 111 223 235 362		10398 10400							2
NC641	M7	086 213	073 146A 199 263 315+C						+		2
NC742	M7	223	073 146 152 189 199 263 315+C	10398 10400	G	C			+		2
NC507	M7b	129 223 297	073 150 199 263 309+CC 315+C						+		2
NC739	M7b	223 297	073 150 199 204 263 315+C						+		2
NC983	M7b	192 223 297	073 131 150 199 266 309+C 315+C						+		2
NC1270	M7b	153 214 223 297	073 150 199 203 204 263 309+C 315+C						+		2
NC1091	M7b	129 223 297	073 150 199 263 315+C 409						+		2
NC785	M7b1	129 192 223 297							+		2
NC825	M7b1	129 192 223 297	073 150 199 263 309+C 315+C						+		2
NC735	M7c	193 223 278 295	073 146 199 263 309+C 315+C	10398 10400					+		2
NC495	M8a1	184 189 212 223 298 319 438									2
NC905	M8a1	184 223 298 319									2
NC935	M8a1	184 189 223 298 311 319 390 443									2
NC994	M8a1	134 184 223 298 319									2
NC455	N9a	223 257A 261 311 362	073 150 263 309+C 315+C								2
NC734	N9a	183C 189 223 257A 261	073 150 263 309+CC 315+C								2
NC749	N9a	129 223 257A 261 284	073 150 263 309+C 315+C								2
NC712	N9a1	111 129 223 257A 261	073 150 263 309+CC 315+C								2
NC1049	R11	178 182C 183C 189 311 390	073 185 234 263 309+CC 315+C								2
NC580	R9b1	192 304 309 390	073 152 195 263 309+C 315+C	CRS							2
NC690	R9b1	082 145 192 243 304 309 390	073 263 315+C	CRS							2
NC1032	R9b1	304 309 390	073 183 263 315+C								2
NC750	R9c	304 335 362	073 150 152 263 309+C 315+C	10403			−	−		+	2
NC633	Z	185 260 298	073 152 195 207 249d 263 309+C 315+C								2

NC661	Z	185 223 260 298	073 152 249d 263 309+C 315+C								2
NC1020	M*	182C 183C 189 223	073 198 215 263 315+C 327	10398 10400	G	C	–	–	+		2
NC710	M*	172 183C 189 209 223 258 311 362	073 185 195 234 263 309+C 315+C								2
NC1365	R*	304 362	073 263 309+C 315+C	CRS	G	C	–	–	+		2

NOTE: Positions are numbered according to the revised Cambridge reference sequence (rCRS) [27]. The sequenced regions that have no mutations compared with the reference sequence are labeled as CRS. When sequence information was not available, items were left blank.

[1] The subjects with and without myopia are abbreviated as M and NC, respectively.

[2] The suffixes "A,G, or C" in the table indicate transversions, "d" indicates a deletion, and a plus sign "+" indicates an insertion. Insertions and deletions are recorded at the last possible site.

[3] e - *Hae* III, g – *Hinf* I, a – *Alu* I. "-" and "+" represent the absence and presence of the respective restriction site.

[4] "1" denotes the presence of 9-bp (CCCCCTCTA) deletion, and "2" denotes non-deletion in the COII/tRNA ^{lys} region.