# ORIGINAL ARTICLE

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# Phylogeographic analysis of mitochondrial DNA haplogroup F2 in China reveals T12338C in the initiation codon of the ND5 gene not to be pathogenic

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Abstract In this report, we studied on a homoplasmic T12338C change in mitochondrial DNA (mtDNA), which substituted methionine in the translational initiation codon of the NADH dehydrogenase subunit 5 gene (ND5) with threonine. This nucleotide change

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was originally identified in two mtDNAs belonging to haplogroup F2 by our previous complete sequencing of 48 mtDNAs. Since then, a total of 76 F2 mtDNAs have been identified by the variations occurring in the hypervariable segments and coding regions among more than 3,000 individuals across China. As the T12338C change was detected in 32 samples representing various sub-clades of the F2 haplogroup while not in 14 non-F2 controls, we believe that the T12338C change is specific to the F2 haplogroup. As F2 and its sub-clades were widely distributed in normal individuals of various Chinese populations, we conclude that T12338C is not pathogenic. In addition, based on the average distribution frequency, haplotype diversity and nucleotide diversity of haplogroup F2 in the populations across China, the T12338C nucleotide substitution seems to have been occurred in north China about 42,000 years ago. Our results provided a good paradigm for distinguishing a polymorphic change from a pathogenic mutation based on mtDNA phylogeny.

**Keywords** Mitochondrial DNA · Haplogroup F2 · T12338C · ND5 · Phylogeny · East Asian

# Introduction

In the past two decades, many mutations, including point mutations, insertions, deletions, and rearrangements, have been detected in protein-coding genes as well as tRNA and rRNA genes in mitochondrial DNA (mtDNA), and were suggested to be pathogenic (Wallace et al. 1999; DiMauro and Schon 2001; Chinnery and Schon 2003). However, among these pathogenic mutations (http://www.mitomap.org), it is rare to find a mutation occurring in the conserved initiation codon of coding genes. Since the change of initiator amino acid from methionine to another

Accession numbers and URLs for the sequence data of mtDNA control region (including HVS-I and HVS-II) of F2 types in this article are as follows: GenBank, http://www.ncbi.nlm.nih.gov/web/ Genbank (accession numbers: AY522667-AY522718).

residue would hamper protein translation, it was natural to regard such a mutation as pathogenic. Hitherto, only three mtDNA mutations have been reported as occurring in the translational initiation codon: T3308C in the ND1 gene (Campos et al. 1997), T7587C in the COX II gene (Clark et al. 1999), and A8527G in the ATP6 gene (Dubot et al. 2004). Mutation T7587C was identified in a family with mitochondrial encephalomyopathy, and suggested to affect translation of COX II (Clark et al. 1999). Mutation T3308C was originally reported to be associated with bilateral striatal necrosis and MELAS (mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes) syndrome (Campos et al. 1997), however, it was later proved to neither impair synthesis of ND1 polypeptide nor affect the activity of complex I, thus suggesting that T3308C might be a benign mutation (Vilarinho et al. 1999; Fernandez-Moreno et al. 2000). Rocha et al. (1999) reached the same conclusion by a phylogenetic analysis. All the mtDNAs harboring T3308C were classified into a particular haplogroup, L1b, which is widely distributed in current African and Iberian populations. The third mutation, A8527G, was recently identified in apparent normal individuals. Although the mutation disrupts the ATG initiation codon of the ATP6 gene, GTG, thus generated, usually encodes valine, may serve as a translational initiation codon in mitochondria (Dubot et al. 2004). The different effects of these mutations in respective cases raise the possibility of some unknown remediation pathway(s). Since the third codon in ND1 is ATG, one of the plausible explanations is that the third codon may serve as an alternative initiation codon to produce similar, but two amino acid short, polypeptide (Rocha et al. 1999; Fernandez-Moreno et al. 2000). Additional mutations and variations occurring in the initiation codon of mtDNA genes in human and other species would clarify the mechanism.

In this study, we report a homoplasmic nucleotide change T12338C in mtDNA, which occurs in the initiation codon of the ND5 gene and substitutes methionine with threonine (M1T). The nucleotide change was originally detected in two mtDNAs (GD7809 and QD8147) when we systematically surveyed mtDNAs belonging to all the major haplogroups specific to East Asian by complete mtDNA sequencing (Kong et al. 2003b). To substantiate whether the T12338C change is specific to haplogroup F2 and to learn more about the origin of haplogroup F2, we performed an extensive search for F2 in more than 3,000 Chinese mtDNAs in reported studies (Yao et al. 2000, 2002a,c, 2003; Tsai et al. 2001; Kivisild et al. 2002; Oota et al. 2002; Yao and Zhang 2002; Kong et al. 2003a; Tajima et al. 2003) and our unpublished data by motif-searching and/or (near-)matching methods (Yao et al. 2002a, 2003). Our phylogeographic study revealed that the T12338C change was specific to haplogroup F2 and occurred in normal individuals across China, thus

suggesting a polymorphic change rather than a pathogenic mutation.

# Material and methods

#### Sampling

A total of 1,494 subjects from 28 populations across China were screened in this study. All of the individuals were confirmed to be unrelated before sampling and were given informed consent. To better understand the phylogeny of haplogroup F2, the previously reported data sets (Yao et al. 2000, 2002a,c, 2003; Tsai et al. 2001; Kivisild et al. 2002; Oota et al. 2002; Yao and Zhang 2002; Kong et al. 2003a; Tajima et al. 2003) were also included. As a result, a total of 3,090 mtDNAs from 57 populations across China were examined, and their detailed information was illustrated in Table 1.

## DNA amplification and sequencing

The hypervariable segments I (HVS-I) and II (HVS-II) of mtDNA control region as well as the regions including potential variations were amplified and sequenced as described elsewhere (Yao et al. 2002a; Kong et al. 2003a,b).

#### Data analyses

The mtDNA sequences were edited and aligned by the DNAStar software package. Mutations were scored according to the revised Cambridge reference sequence (rCRS; Andrews et al. 1999). Length polymorphisms of A and/or C stretches in region 16180-16193 in HVS-I and region 303-315 in HVS-II were disregarded in the subsequent analysis. Each mtDNA was tentatively assigned to a haplogroup on the basis of the variations in the HVS-I and II control regions. The haplogroup status was further confirmed by detecting additional variations in other regions as described in our previous studies (Yao et al. 2002a, 2003; Kong et al. 2003a). A segment covering region 10171-10659 of the rCRS, which was suggested to be informative in defining East Asian specific haplogroups (Yao et al. 2002a), was adopted to specify the phylogenetic status of the F\* or R9\* mtDNAs (the asterisk attached to haplogroups indicates that the sample was not able to be further classified into the sub-clade(s) of the haplogroup) in our previous studies (Yao et al. 2000, 2002a,c, 2003; Yao and Zhang 2002; Kong et al. 2003a) and unpublished data. For those published data sets (not from our laboratory) with only HVS-I and/or HVS-II information available, we recognized the potential F2 types by

Table 1 Frequency of mtDNA haplogroup F2 in Chinese ethnic populations

Population	Abbreviation	Location	No. of samples examined	No. of samples with F2 haplotype	Frequency (%)	References
XJ Han	XJ	Yili, Xinjiang	47	2	4.3	Yao et al. (2002a)
Kazak	Kaz	Xinjiang	30	1	3.3	Yao et al. (2000)
Uygur	Uyg	Xinjiang	45	0	0	Yao et al. (2000)
Huizu	Hui	Yili, Xinjiang	45	1	2.2	This study
Mongolian	Mg	Yili, Xinjiang	49	1	2.0	This study
QH Han	QH	Qinghai	95	1	1.1	This study
QH DM	QHD	Qinghai	78	1	1.3	This study
Tibetan	Tibet	Qinghai	37	2	5.4	This study
Tuzu	Tu	Qinghai	64	2	3.1	This study
Mongolian	Mg	Qinghai	15	1	6.7	Yao et al. (2002c)
Mongolian	Mg	Inner Mongolia	48	1	2.1	Kong et al. (2003a)
Xibe	Xibe	Inner Mongolia	49	0	0	This study
Oroqen	Oro	Inner Mongolia	44	0	0	Kong et al. $(2003a)$
Korean	Kor	Inner Mongolia	48	0	0	Kong et al. $(2003a)$
Ewenki	EWK	Inner Mongolia	4/	0	0	Kong et al. $(2003a)$
Daur	Daur	Inner Mongolia	45	0	0	Kong et al. $(2003a)$
Buryat	Bur	Inner Mongolia	58	0	0	This study
Manchu	Man	Inner Mongolia	12	1	1.4	This study
QD Han	QD	Qingdao, Shandong	50	l	2.0	Y ao et al. $(2002a)$
SD Han	SD	Taran, Shandong	/6	0	7.9	Yao et al. $(2003)$
LN Han		Fengcheng, Liaoning	51	1	2.0	Yao et al. $(2002a)$
Manchu	Man	Fengcheng, Liaoning	30	0	0	I his study $V_{2} = 1 (2002 -)$
Lisu	Lisu	Gongshan, Yunnan	3/	4	10.8	Y ao et al. $(2002c)$
Nuzu	NU	Gongshan, Yunnan	30	5	16.7	Y ao et al. $(2002c)$
Baizu	Bai	Dali, Yunnan	31	1	3.2	Yao et al. $(2002c)$
Lanu	Lanu	Yunnan Liii-n- Mannan	35 50	9	25.7	Yao and Zhang (2002)
INAXI Tibatan	Naxi Tihat	Lijiang, Yunnan	50 40	3	5.4	This study $V_{2,2}$ at al. (2002a)
Tibetan	Tibet	Yunnan, Qingnai	40	0	0	Y ao et al. $(2002c)$
Sani (Tizu)	Sam Honi	Nuxi, Yunnan	25	1	3.2	This study
Lineary	Hani Line	Nuxi, Yunnan	23	0	0	This study
Vucena	JIIIO V o	I uiiiiaii Vunnan	24	0	0	This study
WH Hon		Tulliali Wuhan Hubei	54 42	0	18	$V_{ao}$ et al. (2002a)
OI Hon		Qijijang Chongging	42	2	4.0	This study $(2002a)$
GD Han	GD GD	Zhanijang, Guangdong	30	2 1	13.3	$V_{20}$ et al. (2002a)
GD Han	GD	Chaoshan Guangdong	51	4	0	This study
GD Han	GD	Meizhou Guangdong	61	1	16	This study
GD Han	GD	Guangdong	69	3	43	Kivisild et al. (2002)
VN DM	VND	Kunming Vunnan	07	1	1.0	This study
YN Han	YNC	Kunning, Yunnan	82	2	2.4	This study
YN Han	YN	Kunming, Yunnan	43	1	2.1	Yao et al. $(2002a)$
Vazu	Va	Yunnan	36	0	0	Yao and Zhang (2002)
Daizu	Dai	Xishuangbanna, Yunnan	41	1	2.4	Yao et al. $(2002c)$
SX Han	SX	Xi'an Shaanxi	85	0	0	Oota et al. $(2002)$
HN Han	HN	Changsha, Hunan	82	Ő	ŏ	Oota et al. $(2002)$
Tuija	Ti	Fenghuang, Hunan	46	Õ	Õ	This study
Miaozu	Miao	Fenghuang, Hunan	48	Õ	Õ	This study
Miaozu	Miao	Kaili, Guizhou	35	4	11.4	This study
Shezu	She	Fuquan, Guizhou	54	1	1.9	This study
Shuizu	Shui	Sandu, Guizhou	64	0	0	This study
Bouyei	By	Zhenning, Guizhou	26	0	0	This study
Zhuangzu	Zhuang	Guangxi	83	0	0	Yao et al. (2002c)
Mulam	Mulam	Luocheng, Guangxi	36	2	5.6	This study
Jingzu	Jing	Guangxi	61	5	8.2	This study
Lizu	Li	Hainan	59	2	3.4	This study
TW Han	TW	Taiwan	155	0	0	Tsai et al. (2001)
Taiwanese	Tai	Taiwan	180	0	0	Tajima et al. (2003)
Total			3,090	76		/

matching and/or near-matching with the identified F2 types that have been tested for coding region information. To better understand the relationships among haplogroups, a network profile of haplogroup F2 was constructed according to Bandelt et al. (2000). We also estimated the haplotype diversity and nucleotide diversity (Nei 1987) of haplogroup F2 by using the DnaSP package (Rozas and Rozas 1999). **Table 2** Sequence variations in 76 mtDNAs of haplogroup F2. When the analyzed sequences were identical to the revised Cambridge reference sequence, items are indicated with CRS. When (a) nucleotide change(s) was detected compared to the CRS sequence, only the number of position is indicated for transition, the number with a suffix (i.e. A, C, G, and T) for

GD48 Guangdo GD7810 Zhanjian GD7810 Zhanjian Guangd GD49 Guangdc Kaz28 Xinjiang Miao10 Kaili, Gu Miao5 Tai'an, S ZD10316 Qinghai SD10301 Tai'an, S QJ516 Qinghai WH648 Wuhan, Sh153 Fuquan,	ng g, ong		(± 00001) 1-CALI	HVS-II (30–407) (73, 263, and 315+C in addition) <sup>b</sup>	10171-10659 ( $10000 + )^{c}$	at 12338	References
GD49 Cuangu Kaz28 Guangdo Kaili, Gu Miao5 Kaili, Gu SD10316 Tai'an, S Tibet27 Qinghai SD10301 Tai'an, S QJ516 Qijjang, Mg11 WH6948 Wuhan, Shel 5 Fuquan,	DILG	F2 F2	CRS 261	235 249d 194 235 249d 309 + C	310 535 586	С	Kivisild et al. (2002) Yao et al. (2002a)
Kazzs Annjang Miao10 Kaili, Gu Miao5 Kaili, Gu SD10316 Tai'an, S Tibet27 Qinghai SD10301 Tai'an, S QJ516 Qijiang, <sup>1</sup> Mg11 Qinghai WH6948 Wuhan, Shel5 Fuquan, Man Kan Kan Kan Kan Kan Kan Kan Kan Kan K	ng	F2	209 304	249d 309+C			Kivisild et al. (2002)
Miao5 Kaili, Gu SD10316 Tai'an, S Tibet27 Qinghai SD10301 Tai'an, S QJ516 Qijiang, G Mg11 Qinghai WH6948 Wuhan, Shel 5 Fuquan,	uizhou	F2 F2	304 304	195 249d	310 535 586		Yao et al. (2000) This study
SD10316 Tai'an, S Tibet27 Qinghai SD10301 Tai'an, S QJ516 Qijiang, A Mg11 Qinghai WH6948 Wuhan, She15 Fuquan,	uizhou	F2	304				This study
Tibet27 Qunghai SD10301 Tai'an, S QJ516 Qijiang, A Mg11 Qinghai WH6948 Wuhan, She15 Fuquan,	handong	F2	304	249d 309 + C	310 535 586	C C	Yao et al. (2003)
QJ516 Qijiang, ( QJ516 Qijiang, ( Mg11 Qinghai WH6948 Wuhan, She15 Fuquan, Man153 Inner Mu	معمامهم	F2 F2	304 204 510	PORC	310 535 586 210 525 586		This study
Mgl1 Qinghai WH6948 Wuham She15 Fuquan, Man153 Inner Mu	Chongaing	F2 F2	304 313 304 327 362	249d	310 535 586	50	This study
WH6948 Wuhan, Z She15 Fuquan, Man153 Inner Mi	0	F2	217 221 304		310 535 586	)	Yao et al. (2002c)
She15 Fuquan, Man153 Inner M	Hubei	F2	299 304	249d 309+C	310 535 586	C	Yao et al. (2002a)
Man 53 nor Mc	Guizhou	F2		195 249d 309 + CC	310 535 586		This study
	ongolia	F 2a	18/ 221 291 304	42 + 1 195 249d 275 309 + CC	086 CEC UIE		I his study
YN281 Kunming	. Yunnan	F2a	051 291 304	195 249d	310 535 586		Yao et al. (2002a)
QD8147 <sup>d</sup> Qingdao,	Shandong	F2a1	266 291 304	146 249d	310 535 586	C	Yao et al. (2002a)
SD10335 Tai'an, S	handong	F2a1	266G 291 304 519	249d 309 + C	310 535 586	C	Yao et al. (2003)
SD10340 Tai'an, S	handong	F2a1	266G 291 304 519	249d 309 + CC	310 535 586	S C	Yao et al. (2003)
Tu58 Qinghai		F2a1	266G 291 304 325 519	2493 309 + CC	310 535 586	U C	This study
Naxi80 Lijiang,	Yunnan	F2a1	185 266A 291 304 519	249d	310 535 586	C	This study
Navi72 Lijiang,	Y unnán Vinnán	F 2a 1 F 2a 1	185 266 A 201 304 519	2490 105 2404			This study
Bai21 Dali. Yu:	nnan	F2a1	185 266A 291 304				Yao et al. (2002c)
Nu17 Gongsha	n. Yunnan	F2a1	185 258 266A 291 304				Yao et al. (2002c)
Nu22 Gongsha	n, Yunnan	F2a1	185 258 266A 291 304				Yao et al. (2002c)
Nu26 Gongsha	n, Yunnan	F2a1	185 258 266A 291 304				Yao et al. (2002c)
Nu30 Gongsha	n, Yunnan	F2a1	185 258 266A 291 304				Yao et al. (2002c)
Miaol Kaili, Gu	uizhou	F2a2	092A 291 304				This study
Miao32 Kaili, Gu	iizhou	F2a2	092A 291 304				This study
Mulam13 Luochen	g, Guangxi	F2a2	092A 291 304				This study
GD50 Guanado	g, Guangai ma	Г 242 F 73 2	092A 291 304 092A 291 304	7494			LIIIS Study Kivisild et al (2002)
SD10360 Tai'an S	handono	F2a2 F2a2	002 A 201 304	249d	310 535 586		Yan et al $(2003)$
XJ8414 Yili, Xin	iang	F2a2	092A 291 304	249d 309 + CC	310 535 586	C	Yao et al. (2002a)
QJ534 Qijiang, G	Chongqing	F2a2	092A 291 299 304 362 519	249d 309 + C	310 535 586	C	This study
SD10302 Tai'an, S	handong	F2a2	092A 289 291 304	$249d \ 309 + CC$	310 535 586	C	Yao et al. (2003)
GD7836n Zhanjian	g, Guangdong	F2a2	092A 291 304 359	249d 309 + C	310 535 586		Yao et al. (2002a)
Jing100 Guangxi		F2a2	092A 291 304 311	195 249d		C	This study
Jing126 Guangxi		F2a2	092A 291 304 311	195 249d			This study
Jing140 Guangxi		r za z	092A 291 304 311	195 2490 105 201		C	This study
Linges Guangxi		Г 2а2 Е7а2	092A 291 304 311 007A 201 304 311	195 2490 105 2404		J	This study
Lisu12 Gonosha	n. Yunnan	F2a2 F2a2	092A 170C 189 291 294 304				Yao et al. (2002c)

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Sample <sup>a</sup>	Location	Haplogroup	HVS-I (16000+)	HVS-II (30–407) (73, 263, and 315+C in addition) <sup>b</sup>	10171-10659 (10000 + ) <sup>c</sup>	Nucleotide at 12338	References
Dai51 Lisu34 Lisu45 Lisu30 Nu14	Xishuangbanna, Yunnan Gongshan, Yunnan Gongshan, Yunnan Gongshan, Yunnan Gongshan, Yunnan	F2a2 F2a2 F2a2 F2a2 F2a2	092A 170T 189 291 304 092A 170T 189 291 304 092A 170T 189 291 304 092A 170T 189 291 304 092A 170T 183T 189 291 304 400 092A 170T 183T 189 291 304				Yao et al. (2002c) Yao et al. (2002c)
XJ8407 YND303 Tu40 Hui38 OH9477	Yili, Xinjiang Kumming, Yunnan Qinghai Yili, Xinjiang	F2a3 F2a3 F2a3 F2a3 F2a3 F2a3	203 239 291 304 203 291 304 519 203 291 304 519 203 291 304 519 093 203 291 304 093 203 291 304	249d 309 + C 249d 309 + C 249d 309 + C	310 535 586 310 535 586 310 535 586 310 535 586		Yao et al. (2002a) This study This study This study This study
Mg242 QHD20	Inner Mongolia Qinghai	F2a3 F2a3 F2ta3	126 203 291 304 126 203 291 304 126 120 203 291 304	249d 309 + C	310 535 586		Kong et al. (2003a) This study
GD/842 Mg46 Tibet34	Znanjiang, Guangdong Yili, Xinjiang Qinghai Econology 1 inomine	F2b F2b F2b	129 189 304 203 304 203 304	2012 2490 6017 201	210 222 280 310 535 586 310 535 586	000	Tao et al. (2002a) This study This study Void et al. (2000a)
LIN 7001 YNC13 GD7809 <sup>d</sup>	Fengeneng, Liaoning Kunming, Yunnan Zhanjiang, Guangdong	F2b F2b F2b	129 203 304 093 203 304 519 086 203 304	195 2490 249d 249d	210 232 280 310 535 586 310 535 586	000	Tao et al. (2002a) This study Yao et al. (2002a)
Lahu95 Lahu63 Lahu65 Lahu74 Lahu81 Lahu82	Yunnan Yunnan Yunnan Yunnan Yunnan	F2b F2b F2b F2b F2b F2b	086 167 203 304 519 086 167 203 304 318 086 167 203 304 318 086 167 203 304 318 086 167 203 304 318 086 167 203 304 318				Yao and Zhang (2002) Yao and Zhang (2002)
Lahu84 Lahu87 Lahu88 Sani21 Li39 Li50	Y unnan Y unnan Y unnan Nuxi, Y unnan Hainan Hainan	F2b F2b F2b F2c F2c	086 167 203 304 318 086 167 203 304 318 086 167 203 304 318 086 167 203 304 318 304 527 304 527 304 527	249d 309 + C 249d 309 + C	265 310 535 586 265 310 535 586	00	Yao and Zhang (2002) Yao and Zhang (2002) Yao and Zhang (2002) Yao et al. (2002c) This study This study
GD124 WH6974 YNC105	Meizhou, Guangdong Wuhan, Hubei Kunming, Yunnan	F2c F2c F2c	304 527 192 304 086 304G	249d 249d 151 152 249d 309+C	265 310 325 535 586 265 310 535 586 265 310 535 586 598	000	This study Yao et al. (2002a) This study
<sup>a</sup> The abbre <sup>b</sup> <sup>b</sup> All the san <sup>c</sup> For the inc <sup>d</sup> Samples Q	viation for each sample is inc mples analyzed had 73, 263, i licated samples, region 1017 D8147 and GD7809 were co	dicated in Table and 315+C in a 1–10659 was seq mpletely sequen	1 Iddition to (a) change(s) indicated. Fo puenced in this study except for those for those of al. (2003b)	or a example, GD48 had ' c reported by Yao et al. (2	73, 235, 249d, 263, and 002a)	315+C	

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Table 2 (Continued)

### Results

#### Identification of mtDNA haplogroup F2

As had been revealed by our recent study (Kong et al. 2003b), all the characteristic nucleotide variations for haplogroup F2 were located in the coding regions. Thus, it is generally hard to select F2 mtDNAs solely on the basis of sequence information from the hypervariable segments in the control region, but F2a is easily recognized by the variation at the 16291 position from the other F haplogroups and R9\* (Yao et al. 2002a). By sequencing of the segment 10171-10659, it is easy to distinguish the haplogroup F2 from the rests by nucleotide variations at 10310, 10535, and 10586. As a result, a total of 76 haplogroup F2 mtDNAs were identified (Table 2). Comparing with 19 other mtDNAs belonging to other haplogroups (Table 3), several features are discernible. (1) Five mtDNAs with the variations at both 10310 and 10609 were assigned to

**Table 3** Sequence variations in non-F2 type mtDNAs. When the analyzed sequence was identical to the revised Cambridge reference sequence, items are indicated with CRS. When (a) nucleotide change(s) was detected compared to the CRS sequence, only the

haplogroup F1, and their haplogroup status was further confirmed by the variation at 12406. Two of these had motif 16302-16304-16497-249d, and three others were with motif 16172-16304-249d. (2) Ten mtDNAs bearing only the variation at 10310 in segment 10171– 10659 was assigned to (an) unidentified lineage(s) in F. Seven of them had motif 16207-16304-16399-146-249d, and three others had motif 16218-16304-16311-249d. Further analysis showed that the seven mtDNAs with motif 16207-16304-16399-146-249d were characterized by two specific variations at 12396 and 12408, hence belonging to a new haplogroup designated as "F4." (3) The last four samples (two with motif 16157-16256-16304-16335-236-249d and the other two with motif 16304-16362) were found not to have any variations characteristic to F1, F2, F3 (Kong et al. 2003b), or F4, thus belonging to a new lineage (viz., pre-F) in haplogroup R9. Additional information is needed to further specify the phylogenetic positions of these samples.

number of position is indicated for transition, the number with a suffix (i.e. A, C, G, and T) for transversion, with "d" for deletion and with "+" for insertion. When sequence information was not available, items leave blank

Sample <sup>a</sup>	Location	Haplo- group	HVS-I (16000+)	HVS-II (30–407) (73, 263, and 315 + C in addition) <sup>b</sup>	10171–10659 (10000+) <sup>c</sup>	Nucleotide at 12338	Other polymor- phisms	References
Man10	Fengcheng, Liaoning	F1	302 304 497		310 609	Т	12406	This study
Man17	Fengcheng, Liaoning	F1	302 304 497		310 609	Т	12406	This study
Li26	Hainan	F1	172 187 304 343 519	249d 309+C	310 609	Т	12406	This study
OJ81	Qijiang, Chongqing	F1	172 304 465 519	249d 309 + C	310 609	Т	12406	This study
Zhuang73	Luocheng, Guangxi	F1	172 304 465					Yao et al. (2002c)
GD0091	Chaoshan, Guangdong	F4	207 304 399	146 152 281 249d 309 + C	310	Т		This study
YND617	Kunming, Yunnan	F4	179 207 304 399	146 249d 309 + CC	310	Т		This study
QH9678	Qinghai	F4	207 304 362 399		310	Т		This study
XJ8440	Yili, Xinjiang	F4	207 304 362 399	146 152 249d	310	Т	12396 12408	Yao et al. $(2002a)$
Zhuang30	Guangxi	F4	126 140 207 304 362 399					Yao et al. $(2002c)$
YNC237	Kunming, Yunnan	F4	207 304 362 399 497	146 152 249d	310	Т	12396 12408	This study
YND379	Kunming, Yunnan	F4	093 207 304 362 399 497	$146\ 152\ 207\ 249d$ $309 \pm C$	310	Т	12396 12408	This study
Mulam6	Luocheng, Guangxi	F*	218 304 311	202 0	310	Т		This study
Li56	Hainan	F*	218 304 311 519	$249d \ 309 + C$	310	Ť		This study
Zhuang43	Guangxi	F*	129 218 265 304 311 355					Yao et al. $(2002c)$
Mulam42	Luocheng, Guangxi	R9*	304 362	CRS	CRS	Т		This study
Dai100	Xishuangbanna, Yunnan	R9*	304 362	CRS	CRS	T		Yao et al. $(2002c)$
She61	Fuquan, Guizhou	R9*	157 256 266 304 311 335	236 249d	CRS			This study
YN170	Kunming, Yunnan	R9*	157 256 304 335	236 249d	CRS			Yao et al. (2002a)

<sup>a</sup>The abbreviation for each sample is indicated in Table 1

<sup>b</sup>All the samples analyzed had 73, 263, and 315 + C in addition to (a) change(s) indicated. Mulam42 and Dai100 had 73, 263, and 315 + C, too

<sup>c</sup>For the indicated samples, region 10171–10659 was sequenced in this study except for except for XJ8440 and YN170

## Phylogeny of the haplogroup F2

The network profile of haplogroup F2 revealed that this haplogroup was divided into three major branches, designated as "F2a" (defined by the variation at 16291; Yao et al. 2002a), "F2b" (recognizable by the variation at 10810; Table 4), and "F2c" (characterized by the variation at 10265) (Fig. 1). Haplogroup F2a comprises three major clades, F2a1 (recognizable by the variation at 16266), F2a2 (characterized by a transversion (T/A) at 16092), and F2a3 (defined by the variation at 16203). It is evident that the sub-clades of F2 show regional distribution. For instance, most of the F2b and F2a3 types, as well as all the F2a1 types, are confined to north or north-origin populations. In contrast, individuals belonging to haplogroup F2c are prevalent in south China.

# T12338C is characteristic of haplogroup F2

Our previous analyses of the major haplogroups in East Asian by using complete sequences revealed that the T12338C variation is exclusively detected in two samples of haplogroup F2 (GD7809 and OD8147; Kong et al. 2003b). Extensive search with more than 1,000 published complete mtDNA sequences (Ingman et al. 2000; Finnilä et al. 2001; Maca-Meyer et al. 2001; Torroni et al. 2001; Derbeneva et al. 2002; Herrnstadt et al. 2002; Kivisild et al. 2002 and references therein; Ingman and Gyllensten 2003; Mishmar et al. 2003) revealed two additional cases with the T12338C variation, with one in a P type (Ingman and Gyllensten 2003) and another in a H1 type (Herrnstadt et al. 2002). To further clarify whether the T12338C variation is characteristic of haplogroup F2 or not, 32 samples were selected from our collection described in Table 2 so as to include at least one sample from respective sub-clades, and subjected to PCR amplification and subsequent direct sequencing for detecting the variation. As a control, 14 samples belonging to non-F2 haplogroups selected from the samples listed in Table 3 were also analyzed. Our results revealed that the T12338C nucleotide substitution was detected in all the F2 samples analyzed but not in any non-F2 samples (Tables 2, 3). Further analyses showed that the T12338C variation was completely linked with the T1005C, T1824C, A7828G, T10535C, G10586A, and G13708A variations (Table 4), thus showing T12338C is one of the characteristic variations specific to haplogroup F2.

# Discussion

In this study, we report a homoplasmic nucleotide change, T12338C, which results in substitution of highly conserved methionine at the translation start site of the ND5 gene with threonine (M1T). The T12338C change is tightly associated with other nucleotide variations

Table 4 Relevant nucleotide variations in major sub-clades of haplogroup F2. Only varied nucleotides are indicated with a letter while the identical nucleotide to rCRS is indicated with dots. When information for nucleotide sequences is not available, the items have been left blank

Haplogro	up Representative san	nple Nu(	cleoti	ide va	uriatio	ns at	the ir	ıdicatε	sod pa	ition																
		100	5 170	9 182	24 329	<del>)</del> 0 481	1 541	7 584	6 6392	2 7828	8636	9137	1031(	) 1053	5 105	36 108	01 108	10 123	38 126	09 123	66 13	500 13	708 139	28 14(	16 1450	50
H	rCRS	Τ	IJ.	Ε	Ε	A	ŋ	C	L	Ā	Ĺ	L	Ŀ	Ţ	IJ.	ŋ	Τ	Ţ	Ţ	Y	Γ	IJ.	ŋ	ŋ.	G	
F2a1 F2a1	QD8147 Tu58	υu	◀ .	υu	υ.	ს.	V.	Η.	00	ს ს	υ.	υ.	<  <	υu	< <	V.	•	υu	υ.	•	υ.	<b>4 4</b>	00	₹.	•	
F2a1	Naxi80	C		C	•		•		C	Ċ			A	C	A	•		C	•		•	A	C	•		
F2a2	XJ8414	U		C	•	Ċ	•		U	Ċ			V	υ	V	•		U				V	U			
F2a3	XJ8407	U		C			•	•	U	Ċ			V	U	V	•		U	•		•	A	U	•		
F2b	GD7809	C		C	•				U	Ċ			A	U	A		U	U		Ċ		A	U		A	
F2b	Mg46	U		U						Ċ			A	U	A		U	U				A	U		A	
F2b	LN7601	U		C						Ċ			A	U	A		U	U				A	U			
F2b	GD7842	U		U						Ċ			V	U	V		U	U				A	U			
F2a3	Mg242	U		C						Ċ			A	U	A		•	U				A	U			
F2a3	QH9477	U		U						Ċ			V	U	V			U				A	U			
F2a3	Hui38	U		C						Ċ			V	U	V			U		•		A	U			
F2c	WH6974	U		U						Ċ			A	υ	A		•	U				A	U			
F2	WH6948	U		U						Ċ			A	U	A		•	U		•		A	U			
F2	GD7810	C		C						Ċ			A	C	A			C				Α	C			

Fig. 1 Network profile of haplogroup F2 samples observed in Chinese. The network is constructed on the basis of 76 F2 mtDNAs identified in 57 Chinese ethnic populations. As the nucleotide at site 16519 is known to be extremely hypervariable, it is disregarded in the construction. Representative sample names are indicated in the circles, and the circle size is proportional to the number of samples to be belonged among the pool. Relevant nucleotide variations compared with the revised Cambridge Reference Sequence (rCRS) indicate on the branches in the number of the position. Recurrent mutations are underlined, and the asterisk indicates the root of haplogroup F2



including T1005C, T1824C, A7828G, T10535C, G10586A, and G13708A. Thus, these variations together characterize the F2 haplogroup. Considering the fact that mitochondria of haplogroup F2 was distributed widely in normal populations across China though with relatively low frequencies (Table 1), and no evidence suggesting any association of the haplogroup with mitochondrial disorders, it is clear that T12338C is a polymorphic variation rather than a pathogenic mutation. This case is similar to that of T3308C, which is specific to haplogroup L1b and has been proven to be benign (Rocha et al. 1999; Fernandez-Moreno et al. 2000). Intriguingly, since the third codon of the ND5 gene also encodes methionine, similar to that of the ND1 gene, it is plausible that the third codon of the ND1 and ND5 genes would act as a surrogate when the initiation codon was impaired (Rocha et al. 1999; Fernandez-Moreno et al. 2000). In the COX II gene, the second methionine codon in frame is located 48 nucleotides downstream of the translational initiation site. Even though this codon could take a similar proxy role as discussed for the ND1 and ND5 genes, it is still uncertain whether the resultant polypeptide with 16 residues shorter is functional. Thus, it is not surprising that a similar phenomenon caused by T7587C in the COX II gene leads to a mitochondrial disorder (Clark et al. 1999). Nucleotide changes occurring in the translational initiation codon are also observed in mtDNAs of primates (Table 5). Two and four cases have been reported for the ND1 and ND5 genes, respectively, in chimpanzees (Horai et al. 1995), macaques, and patas (Hayasaka et al. 1996). It seems that the first codon in proposed cDNA sequence for the ND1 and ND5 genes are not crucial, and the adjacent downstream ATG in frame could take a role in translational initiation.

Haplogroup is normally of higher prevalence and has more genetic diversity at the place it occurred than the radiated area. To speculate where the F2 haplogroup (viz., T12338C) originated, we simply aggregated the data form respective populations into northern and southern groups according to their locations and/or ethono-origin (we did not include samples WH Han and OJ Han because they were located in the Changiang River region where populations migrate considerably). Although this strategy has been criticized (Yao et al. 2002a), this method would enlarge the sample size and make the comparison less affected by the sample size bias. A slightly higher frequency of haplogroup F2 was identified in the northern group (45/1,473) than in the southern group (27/1,524). The haplogroup diversity and nucleotide diversity in F2 are slightly higher in the northern group  $(0.940 \pm 0.021 \text{ and } 0.0106 \pm 0.0006)$ , respectively) than those in the southern group  $(0.915 \pm 0.033 \text{ and } 0.0075 \pm 0.0008$ , respectively). These results suggest that the F2 haplogroup might originate in north China. This suggestion is also supported by two

Changes in nucleotide sequences	Changes in amino acid sequences	Gene	Accession number	Organism	References
$ATA \rightarrow ACA$	$\mathrm{MPM} \to \mathrm{TPM}$	ND1	NA	Homo sapiens	Campos et al. (1997), Fernandez-Moreno et al. (2000), Polyak et al. (1998), Vilarinho et al. (1999), Rocha et al. (1999) and Opdal et al. (2002)
$ATA \rightarrow AGA$	$\mathbf{M} \rightarrow \text{termination}$	ND1	NA	Homo sapiens	Opdal et al. (2002)
$ATG \rightarrow ACG$	$MAH \rightarrow TAH$	COX II	NA	Homo sapiens	Clark et al. (1999)
$ATG \rightarrow GTG$	$MNE \rightarrow VNE$	ATP6	AY370877	Homo sapiens	Dubot et al. (2004)
$ATA \rightarrow ACA$	$MTM \rightarrow TTM$	ND5	AY255168	Homo sapiens	Kong et al. (2003b)
$ATA \rightarrow ACA$	$MTM \rightarrow TTM$	ND5	AY255180	Homo sapiens	Kong et al. (2003b)
$ATA \rightarrow ACA$	$MTM \rightarrow TTM$	ND5	AY289092	Homo sapiens	Ingman and Gyllensten (2003)
$ATA \rightarrow ACA$	$MTM \rightarrow TTM$	ND5	NA	Homo sapiens	Herrnstadt et al. (2002)
$ATA \rightarrow ACA$	$MPM \rightarrow TPM$	ND1	NC 001643	Pan troglodytes	Horai et al. (1995)
$ATA \rightarrow ACA$	$MPM \rightarrow TPM$	ND1	NC_001644	Pan paniscus	Horai et al. (1995)
$ATA \rightarrow ACA$	$MIM \rightarrow TIM$	ND5	D85291	Erythrocebus patas	Hayasaka et al. (1996)
$ATA \rightarrow ACA$	$MIM \rightarrow TIM$	ND5	D85287	Macaca nigra	Hayasaka et al. (1996)
$ATA \rightarrow GTA$	$MIM \rightarrow VIM$	ND5	D85285	Macaca nemestrina	Hayasaka et al. (1996)
$ATA \rightarrow GTA$	$\mathrm{MIM} \to \mathrm{VIM}$	ND5	D85286	Macaca silenus	Hayasaka et al. (1996)

Table 5 Mutations and/or variations occurring in the initiation codon of mitochondria genes. NA, not available

features in the phylogeographic analysis of F2 (Fig. 1). (1) The potential root types of F2 were more prevalent in the populations from north China or of the northern origin. (2) Almost all the major sub-clades of F2 were distributed in the northern populations except for F2c (which is exclusive to south China). By counting the transitions in region 16090–16365 (Forster et al. 1996; Saillard et al. 2000), we calculated the age of haplogroup F2 to be  $41,700 \pm 13,700$  years. These results suggested that haplogroup F2 might originate and expand in north China before the last Glacier Age. The prevalence of sub-haplogroups of F2, for examples F2c and F2a2, in south China might reflect back-migration events from north China to south China afterwards (Yao et al. 2002a).

Our current study also raises a concern in identifying pathogenic mtDNA mutations. Several surveys have revealed that mutation C5178A is associated with longevity and other disorders (Kokaze et al. 2003 and references therein). According to our study, the C5178A variation is exclusive associated with haplogroup D (Yao et al. 2002b), and it is well known that haplogroup D is prevalent in northern Chinese (Yao et al. 2002a; Kong et al. 2003a) and Japanese (Maruyama et al. 2003). Thus, the most results of association of C5178A with, i.e., a disease phenotype would rather reflect the existence of population stratification (Ardlie et al. 2002) and/or inadequate sampling. Another paper reporting an association of G15497A with obesity (Okura et al. 2003) comprises a similar case, since G15497A together with T8200C and G15323A are characteristic to haplogroup G1, a prevalent form in northeastern Asia (Bandelt et al. 2003; Kong et al. 2003b). Such a hasty conclusion in association studies between nucleotide changes in mtDNA with disorders could be avoidable if the authors should refer to phylogenetic information of mtDNA.

In conclusion, our mutational and phylogeographical analyses with human mtDNA indicate that the T12338C change is one of the characteristic variations associated with haplogroup F2, thus polymorphic, and not a pathogenic mutation.

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#### References

- Andrews RM, Kubacka I, Chinnery PF, Lightowlers RN, Turnbull DM, Howell N (1999) Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. Nat Genet 23:147
- Ardlie KG, Lunetta KL, Seielstad M (2002) Testing for population subdivision and association in four case-control studies. Am J Hum Genet 71:304–311
- Bandelt H-J, Macaulay V, Richards M (2000) Median networks: speedy construction and greedy reduction, one simulation, and two case studies from human mtDNA. Mol Phylogenet Evol 16:8–28
- Bandelt H-J, Herrnstadt C, Yao Y-G, Kong Q-P, Kivisild T, Rengo C, Scozzari R, Richards M, Villems R, Macaulay V, Howell N, Torroni A, Zhang Y-P (2003) Identification of native American founder mtDNAs through the analysis of complete mtDNA sequences: some caveats. Ann Hum Genet 67:512–524
- Campos Y, Martin MA, Rubio JC, Gutierrez del Olmo MC, Cabello A, Arenas J (1997) Bilateral striatal necrosis and MELAS associated with a new T3308C mutation in the mitochondrial ND1 gene. Biochem Biophys Res Commun 238:323–325
- Chinnery PF, Schon EA (2003) Mitochondria. J Neurol Neurosurg Psychiatry 74:1188–1199
- Clark KM, Taylor RW, Johnson MA, Chinnery PF, Chrzanowska-Lightowlers ZM, Andrews RM, Nelson IP, Wood NW, Lamont PJ, Hanna MG, Lightowlers RN, Turnbull DM (1999) An mtDNA mutation in the initiation codon of the cytochrome C oxidase subunit II gene results in lower levels of the protein and a mitochondrial encephalomyopathy. Am J Hum Genet 64:1330–1339
- Derbeneva OA, Sukernik RI, Volodko NV, Hosseini SH, Lott MT, Wallace DC (2002) Analysis of mitochondrial DNA diversity in the aleuts of the commander islands and its implications for the genetic history of beringia. Am J Hum Genet 71:415–421

- DiMauro S, Schon EA (2001) Mitochondrial DNA mutations in human disease. Am J Med Genet 106:18–26
- Dubot A, Godinot C, Dumur V, Sablonniere B, Stojkovic T, Cuisset JM, Vojtiskova A, Pecina P, Jesina P, Houstek J (2004) GUG is an efficient initiation codon to translate the human mitochondrial ATP6 gene. Biochem Biophys Res Commun 313:687–693
- Fernandez-Moreno MA, Bornstein B, Campos Y, Arenas J, Garesse R (2000) The pathogenic role of point mutations affecting the translational initiation codon of mitochondrial genes. Mol Genet Metab 70:238–240
- Finnilä S, Lehtonen MS, Majamaa K (2001) Phylogenetic network for European mtDNA. Am J Hum Genet 68:1475–1484
- Forster P, Harding R, Torroni A, Bandelt H-J (1996) Origin and evolution of Native American mtDNA variation: a reappraisal. Am J Hum Genet 59:935–945
- Hayasaka K, Fujii K, Horai S (1996) Molecular phylogeny of macaques: implications of nucleotide sequences from an 896base pair region of mitochondrial DNA. Mol Biol Evol 13:1044–1053
- Herrnstadt C, Elson JL, Fahy E, Preston G, Turnbull DM, Anderson C, Ghosh SS, Olefsky JM, Beal MF, Davis RE, Howell N (2002) Reduced-median-network analysis of complete mitochondrial DNA coding-region sequences for the major African, Asian, and European haplogroups. Am J Hum Genet 70:1152–1171
- Horai S, Hayasaka K, Kondo R, Tsugane K, Takahata N (1995) Recent African origin of modern humans revealed by complete sequences of hominoid mitochondrial DNAs. Proc Natl Acad Sci USA 92:532–536
- Ingman M, Gyllensten U (2003) Mitochondrial genome variation and evolutionary history of Australian and New Guinean aborigines. Genome Res 13:1600–1606
- Ingman M, Kaessmann H, Paabo S, Gyllensten U (2000) Mitochondrial genome variation and the origin of modern humans. Nature 408:708–713
- Kivisild T, Tolk HV, Parik J, Wang Y, Papiha SS, Bandelt H-J, Villems R (2002) The emerging limbs and twigs of the East Asian mtDNA tree. Mol Biol Evol 19:1737–1751
- Kokaze A, Ishikawa M, Matsunaga N, Yoshida M, Sekine Y, Sekiguchi K, Satoh M, Harada M, Teruya K, Takeda N, Uchida Y, Tsunoda T, Takashima Y (2003) Longevity-associated mitochondrial DNA 5178 A/C polymorphism modulates effects of daily drinking and cigarette consumption on serum triglyceride levels in middle-aged Japanese men. Exp Gerontol 38:1071–1076
- Kong Q-P, Yao Y-G, Liu M, Shen S-P, Chen C, Zhu C-L, Palanichamy MG, Zhang Y-P (2003a) Mitochondrial DNA sequence polymorphisms of five ethnic populations from northern China. Hum Genet 113:391–405
- Kong Q-P, Yao Y-G, Sun C, Bandelt H-J, Zhu C-L, Zhang Y-P (2003b) Phylogeny of East Asian mitochondrial DNA lineages inferred from complete sequences. Am J Hum Genet 73:671– 676
- Maca-Meyer N, González AM, Larruga JM, Flores C, Cabrera VC (2001) Major genomic mitochondrial lineages delineate early human expansions. BMC Genetics 2:13
- Maruyama S, Minaguchi K, Saitou N (2003) Sequence polymorphisms of the mitochondrial DNA control region and phylogenetic analysis of mtDNA lineages in the Japanese population. Int J Legal Med 117:218–225
- Mishmar D, Ruiz-Pesini E, Golik P, Macaulay V, Clark AG, Hosseini S, Brandon M, Easley K, Chen E, Brown MD, Sukernik RI, Olckers A, Wallace DC (2003) Natural selection shaped regional mtDNA variation in humans. Proc Natl Acad Sci USA 100:171–176

- Nei M (1987) Molecular evolutionary genetics. Columbia University, New York
- Okura T, Koda M, Ando F, Niino N, Tanaka M, Shimokata H (2003) Association of the mitochondrial DNA 15497G/A polymorphism with obesity in a middle-aged and elderly Japanese population. Hum Genet 113:432–436
- Oota H, Kitano T, Jin F, Yuasa I, Wang L, Ueda S, Saitou N, Stoneking M (2002) Extreme mtDNA homogeneity in continental Asian populations. Am J Phys Anthropol 118:146–153
- Opdal SH, Vege A, Egeland T, Musse MA, Rognum TO (2002) Possible role of mtDNA mutations in sudden infant death. Pediatr Neurol 27:23–29
- Polyak K, Li Y, Zhu H, Lengauer C, Willson JK, Markowitz SD, Trush MA, Kinzler KW, Vogelstein B (1998) Somatic mutations of the mitochondrial genome in human colorectal tumours. Nat Genet 20:291–293
- Rocha H, Flores C, Campos Y, Arenas J, Vilarinho L, Santorelli FM, Torroni A (1999) About the "Pathological" role of the mtDNA T3308C mutationellipsis. Am J Hum Genet 65:1457– 1459
- Rozas J, Rozas R (1999) DnaSP version 3: an integrated program for molecular population genetics and molecular evolution analysis. Bioinformatics 15:174–175
- Saillard J, Forster P, Lynnerup N, Bandelt H-J, Norby S (2000) mtDNA variation among Greenland Eskimos: the edge of the Beringian expansion. Am J Hum Genet 67:718–726
- Tajima A, Sun CS, Pan IH, Ishida T, Saitou N, Horai S (2003) Mitochondrial DNA polymorphisms in nine aboriginal groups of Taiwan: implications for the population history of aboriginal Taiwanese. Hum Genet 113:24–33
- Torroni A, Rengo C, Guida V, Cruciani F, Sellitto D, Coppa A, Calderon FL, Simionati B, Valle G, Richards M, Macaulay V, Scozzari R (2001) Do the four clades of the mtDNA haplogroup L2 evolve at different rates? Am J Hum Genet 69:1348–1356
- Tsai LC, Lin CY, Lee JC, Chang JG, Linacre A, Goodwin W (2001) Sequence polymorphism of mitochondrial D-loop DNA in the Taiwanese Han population. Forensic Sci Int 119:239–247
- Vilarinho L, Chorao R, Cardoso ML, Rocha H, Nogueira C, Santorelli FM (1999) The ND1 T3308C mutation may be a mtDNA polymorphism. Report of two Portuguese patients. J Inherit Metab Dis 22:90–91
- Wallace DC, Brown MD, Lott MT (1999) Mitochondrial DNA variation in human evolution and disease. Gene 238:211–230
- Yao Y-G, Zhang Y-P (2002) Phylogeographic analysis of mtDNA variation in four ethnic populations from Yunnan Province: new data and a reappraisal. J Hum Genet 47:311–318
- Yao Y-G, Lu X-M, Luo H-R, Li W-H, Zhang Y-P (2000) Gene admixture in the silk road region of China: evidence from mtDNA and melanocortin 1 receptor polymorphism. Genes Genet Syst 75:173–178
- Yao Y-G, Kong Q-P, Bandelt H-J, Kivisild T, Zhang Y-P (2002a) Phylogeographic differentiation of mitochondrial DNA in Han Chinese. Am J Hum Genet 70:635–651
- Yao Y-G, Kong Q-P, Zhang Y-P (2002b) Mitochondrial DNA 5178A polymorphism and longevity. Hum Genet 111:462–463
- Yao Y-G, Nie L, Harpending H, Fu Y-X, Yuan Z-G, Zhang Y-P (2002c) Genetic relationship of Chinese ethnic populations revealed by mtDNA sequence diversity. Am J Phys Anthropol 118:63–76
- Yao Y-G, Kong Q-P, Man X-Y, Bandelt H-J, Zhang Y-P (2003) Reconstructing the evolutionary history of China: a caveat about inferences drawn from ancient DNA. Mol Biol Evol 20:214–219