GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Mitochondrial DNA haplogroups and somatic mutations are associated with lung cancer in patients from Southwest China

Y. Fang[1]*, H.Y. Yang[2]*, Y.H. Shi[3], J.H. Cui[4], L.Y. Li[5], Y.C. Xu[5] and J.L. Shao[1]

[1]Department of Anesthesiology,
The First Affiliated Hospital of Kunming Medical University,
Kunming, Yunnan Province, China
[2]Kunming Maternal and Child Health Center, Kunming,
Yunnan Province, China
[3]Clinical Laboratory Department, Cancer Hospital of Shanxi,
Taiyuan, Shanxi, China
[4]Department of Medical Service, PLA Unit 65176, Dalian, Liaoning, China
[5]Department of Cardiology and Clinical Laboratory Department,
211th Hospital of the PLA, Harbin, Heilongjiang, China

*These authors contributed equally to this study.
Corresponding author: J.L. Shao
E-mail: jianlinshaoydyy@163.com

Genet. Mol. Res. 14 (2): 5031-5043 (2015)
Received June 23, 2014
Accepted October 9, 2014
Published May 12, 2015
DOI http://dx.doi.org/10.4238/2015.May.12.6

ABSTRACT. Mitochondrial DNA mutations play crucial roles in the pathogenesis and progression of human malignancies. Therefore, to determine whether maternal background or mitochondrial DNA somatic mutations were essential cofactors in the lung cancer of Chinese patients as well, the complete mitochondrial DNA displacement loop of the primary cancerous, matched para-cancerous normal and distant normal tissues for 79 Chinese patients with lung cancer were analyzed in this study. Our results indicated that the higher detected frequency of haplogroups prevalent in southern East Asia (53.16%; 42/79) versus

those of northern East Asia in the studied population supported the southern East Asian characteristics of the Chinese lung cancer group. Further statistical analysis revealed that the haplogroups F* and G* contributed to the susceptibility to lung cancer in Chinese patients. In addition, by comparing sequences from different tissues of the same patients, a total of eight somatic mutations from six patients were detected. Combined with the fourteen somatic mutations identified in our previous study, the somatic mutation spectrum of the 79 Chinese patients with lung cancer was 25.32% (20/79). Our results suggest that mitochondrial DNA haplogroups and somatic mutations are associated with lung cancer in patients from Yunnan, Southwest China, and that somatic mitochondrial DNA mutations in the displacement loop can serve as potential biomarkers for clinical utility.

Key words: Lung cancer; Mitochondrial DNA; Haplogroup; Somatic mutation

INTRODUCTION

Lung cancer is the leading cause of cancer deaths globally, accounting for 13% of all deaths (Yang Ai et al., 2013) and 30% of all cancer-related deaths (Ferlay et al., 2010; Cao et al., 2011). Lung cancer has also caused approximately 400,000 deaths annually in China (Yang et al., 2009), with late diagnosis and suboptimal therapies being considered the main cause of the low 5-year survival rate in lung cancer.

Mitochondria serve as the crucial intracellular organelle responsible for regulating cellular energy metabolism, producing free radicals, and initiating and executing apoptotic pathways (Wallace, 1995). Much evidence has supported the association between mitochondrial DNA (mtDNA) mutations and various diseases, with somatic mutations in particular having been correlated with various tumors (Wallace, 2005).

Maternally inherited variations in mtDNA have been considered the result of adaptations in the ancestors of modern humans to habitation in cold climatic environments (Mishmar et al., 2003; Ruiz-Pesini et al., 2004). They have also been associated with bioenergetic or mitochondrial dysfunction (Mishmar et al., 2003; Ruiz-Pesini et al., 2004; Wallace, 2005), with previous studies demonstrating associations between mutations of haplogroup N9a and type 2 diabetes in Asians (Fuku et al., 2007) and between haplogroups M7b1'2 and M8 with the expression of Leber hereditary optic neuropathy in Chinese families with the m11778G/A mutation (Ji et al., 2008). mtDNA is highly susceptible to mutation because of its continuous exposure to high levels of reactive oxygen species generated during oxidative phosphorylation (Wallace et al., 1999), leading to a higher mutation rate of mtDNA than of nuclear DNA (Yakes and Van Houten, 1997). Such mutations, which occur in individual cells are not inherited from a parent, and are not passed on to offspring, are called somatic mutations. Furthermore, given the paucity of spacer regions between human mitochondrial genes, a mutation in the mtDNA will most likely involve a functionally important region of the mitochondrial genome. Increasing numbers of studies have revealed that somatic mtDNA mutation may be involved in carcinogenesis and tumor progression. These mutations have been detected with relatively higher frequency by comparison between the primary cancerous, matched para-cancerous

normal, and distant normal tissues from the same patients (Brandon et al., 2006; Chatterjee et al., 2006). To date, a large number of somatic mutations have been detected among various kinds of tumor tissues, such as in breast cancer (Wang et al., 2007; Alhomidi et al., 2013), gastric cancer (Hung et al., 2010), esophageal squamous cell carcinoma (Kumimoto et al., 2004), lung cancer (Jin et al., 2007; Wang and Zhao, 2011; Fang et al., 2013; Yang Ai et al., 2013), and in aging individuals (Williams et al., 2013). Our previous study also revealed elevated somatic mutation rates through the examination of thirty entire mtDNA genomes from ten Chinese patients with lung cancer (Fang et al., 2013), as well as the poly-C repeat stretch (D310) of 79 patients (Chen et al., 2014). Therefore, both germline and somatic mtDNA mutations have been deemed to play crucial roles in tumorigenesis.

Human mtDNA is a circular, double-stranded molecule comprised of approximately 16,569 bp. It contains a displacement loop (D-loop) and 37 genes, coding for 12S and 16S rRNAs, 22 tRNAs, and 13 polypeptides (Anderson et al., 1981; Andrews et al., 1999). The mtDNA D-loop, a 1124-bp long fragment (from position 16,024 to 576) (NC_012920) (Andrews et al., 1999), is a non-coding region, and acts as a promoter for both the heavy and light strands of the mtDNA, containing essential transcription and replication elements. It also contains two hypervariable regions (HVI at position 16,024 to 16,383; and HVII at 57 to 372) (NC_012920) (Andrews et al., 1999), which have consequently been widely used in forensic analyses and medical diagnosis (Sharma et al., 2005; Li et al., 2012). Therefore, we speculated that the variation of the mtDNA D-loop region in Chinese patients with lung cancer would better illustrate the crucial role that mtDNA might play in the mechanism of lung carcinogenesis.

In this study, to determine whether the maternal haplogroups or mtDNA somatic mutations played crucial roles in Chinese patients with lung cancer, both the germ line and somatic mutations of the mtDNA D-loop were identified in 237 samples from 79 Chinese patients with lung cancer; our previously reported D310 mutations were also analyzed.

MATERIAL AND METHODS

Tissue specimens

A total of 237 tissue samples, including the primary lung cancerous tissue, corresponding para-cancerous normal tissue, and distant normal tissue, were collected from 79 patients with lung cancer who received treatment at the First Affiliated Hospital of Kunming Medical University or the Second People's Hospital of Yunnan Province between 2011 and 2012. The primary cancerous and para-cancerous normal tissues were sampled by manual microdissection from hematoxylin and eosin-stained slides. The different tissues from the same individual were marked with suffix "A", "B", or "C", respectively; "A" referred to the para-cancerous normal tissue, "B" referred to the primary lung cancerous tissue, and "C" referred to the distant normal tissue (blood). All procedures were supervised and approved by the human tissue research committee of the First Affiliated Hospital of Kunming Medical University, and informed consents were obtained from all participants.

DNA extraction, polymerase chain reaction (PCR) amplification and sequence analysis

The genomic DNA was extracted using standard phenol/chloroform methodology, and stored at -20°C for future use. The mtDNA D-loop regions (spanning nucleotide posi-

tions 16024-16569/1-576) were amplified and sequenced as fully described in Zhao et al. (2009). Mutations were recorded by comparison with the revised Cambridge reference sequence (rCRS) (Andrews et al., 1999). All subjects were allocated into a specific haplogroup based on their control-region information according to the updated worldwide phylogenetic tree constructed with the entire mtDNA genome (van Oven and Kayser, 2009). Principle component analysis (PCA) was conducted as described previously (Yao et al., 2002a) by taking the haplogroup frequencies of the lung cancer group and of healthy populations as input factors. Statistical analysis was conducted using the SPSS 13.0 software package (SPSS, Chicago, IL, USA), with $P < 0.05$ as a statistically significant difference.

Cloning and sequencing of mtDNA somatic mutations

Cloning and sequencing analysis were performed to verify the authenticity of the somatic mutations detected in the mtDNA D-loop region among Chinese patients with lung cancer. The PCR products of the newly extracted DNA from primary lung cancerous tissues and distant normal tissues in patients with somatic mutations were purified and transferred to the pUC18 vector. The clones were selected and sequenced directly with forward and reverse vector primers, and the sequences were compared with the rCRS (Andrews et al., 1999).

RESULTS

As shown in Table 1, all the lung cancer patients investigated from Southwest China were allocated to haplogroups prevalent in East Asia, Southeast Asia, West Eurasia, or South Asia. The East and Southeast Asian prevalent haplogroups accounted for a significantly higher proportion (96.20%; 76/79) of the patients than did the West Eurasian and South Asian predominant haplogroups (2.53% and 1.27%, respectively). Among the former, the southern East Asian and Southeast Asian prevalent haplogroups, such as B, F, M7, M9, M71, M*, R9, and R11 (Wen et al., 2004a; Kong et al., 2011), accounting for 50.63% (40/79) of the East and Southeast Asian prevalent haplogroups, which was more than the northern East Asian prevalent haplogroups, such as A, C, Z, D, G and N9, which accounted for 44.30% (35/79) of the patients.

To evaluate the association of mtDNA haplogroups with the lung cancer population, the mtDNA variations of 490 healthy individuals from 11 Yunnan ethnic and Han populations were retrieved from the literature (Yao et al., 2002b; Wen et al., 2004a,b, 2005; Zhao et al., 2009). As PCA has been shown to be a powerful tool for detecting true associations in mitochondrial medical genetics (Biffi et al., 2010), we constructed a PCA map by taking the haplogroup frequencies of 79 lung cancer patients and 490 healthy individuals from 11 ethnic and Han groups as input factors. Our results showed that the lung cancer group was clustered with the Naxi, Pumi, and Hani ethnic groups from Yunnan Province. They did not show any separated cluster pattern, as shown in Figure 1, supporting that there was no essential difference between them, and excluding the possibility of population stratification between the lung cancer population and other 11 control groups (including 1 Han and 10 ethnic groups). Further, to confirm whether there was any specific mtDNA haplogroup associated with lung cancer, the statistically significant differences were estimated between the mtDNA haplogroup frequencies of the lung cancer group and healthy populations by pooling the 1 Han Chinese and 10 ethnic groups from different regions of Yunnan as control population for analysis in order to avoid the biasing of results because of small sample size and sampling location.

Table 1. mtDNA mutations in 237 samples from 79 patients with lung cancer from Yunnan, Southwest China.

Sample	Haplogroups	HVSI (16000+)	HVSII	Readable region
1A	M7	129 223 297	73 150 159 199 263 315+C 489	16013-16569/1-554
1B	M7	129 223 297	73 150 159 199 263 315+C 489	16012-16569/1-549
1C	M7	129 223 297	73 150 159 199 263 315+C 489	16018-16569/1-590
2A	D4	223 362	73 152 195 263 315+C 489	16013-16569/1-531
2B	D4	223 362	73 152 195 263 315+C 489	16013-16569/1-588
2C	D4	223 362	73 152 195 263 315+C 489	16012-16569/1-587
3A	D4e	092 223 362	73 94 263 309+C 315+C 489	16012-16569/1-542
3B	D4e	092 223 362	73 94 263 309+C 315+C 489	16014-16569/1-588
3C	D4e	092 223 362	73 94 263 309+C 315+C 489	16013-16569/1-588
4A	F1	189 304 519	73 146 249d 263 309+C 315+C 522-523d	16016-16569/1-542
4B	F1	189 304 519	73 146 249d 263 309+C 315+C 522-523d	16012-16569/1-587
4C	F1	189 304 519	73 146 249d 263 309+C 315+C 522-523d	16012-16569/1-549
5A	F	260 298 355 362	73 249d 263 309+C 315+C 709	16016-16569/1-717
5B	F	260 298 355 362	73 249d 263 309+C 315+C	16016-16569/1-614
5C	F	260 298 355 362	73 249d 263 309+C 315+C	16013-16569/1-499
6A	D4a	129 223 362 519	73 152 217 263 309+2C 315+C 489	16016-16569/1-543
6B	D4a	129 223 362 519	73 152 217 263 309+2C 315+C 489	16013-16569/1-560
6C	D4a	129 223 362 519	73 152 217 263 309+2C 315+C 489	16012-16569/1-554
7A	B4c	147 182+C 189 217 235 519	73 146 263 **309+2C** 315+C	16014-16569/1-581
7B	B4c	147 182+C 189 217 235 519	73 146 263 **309+C** 315+C	16017-16569/1-562
7C	B4c	147 182+C 189 217235 519	73 146 263 **309+2C** 315+C	16017-16569/1-563
8A	F1a1a	129 162 172 304 311 519	73 152 249d 263 315+C 477 522-523d	16011-16569/1-598
8B	F1a1a	129 162 172 304 311 519	73 152 249d 263 315+C 477 522-523d	16012-16569/1-588
8C	F1a1a	129 162 172 304 311 519	73 152 249d 263 315+C 477 522-523d	16013-16569/1-587
9A	D	172 223 362	73 263 309+C 315+C 489	16012-16569/1-585
9B	D	172 223 362	73 263 309+C 315+C 489	16009-16569/1-588
9C	D	172 223 362	73 263 309+C 315+C 489	16012-16569/1-580
10A	R9b	192 304 309 390 519	73 152 263 309+C 315+C	16015-16569/1-553
10B	R9b	192 304 309 390 519	73 152 263 309+C 315+C	16012-16569/1-555
10C	R9b	192 304 309 390 519	73 152 263 309+C 315+C	16018-16569/1-552
11A	B4a	181C 182C 183C 189 213 217 261 519	61A 62 73 263 315+C 522-523d	16012-16569/1-572
11B	B4a	181C 182C 183C 189 213 217 261 519	61A 62 73 263 315+C 522-523d	16013-16569/1-590
11C	B4a	181C 182C 183C 189 213 217 261 519	61A 62 73 263 315+C 522-523d	16013-16569/1-589
12A	M*	129 223 287 311 327A	64 73 93 189 200 263 **309+2C** 315+C 485 489	16012-16569/1-588
12B	M*	129 223 287 311 327A	64 73 93 189 200 263 **309+C** 315+C 485 489	16013-16569/1-555
12C	M*	129 223 287 311 327A	64 73 93 189 200 263 **309+2C** 315+C 485 489	16014-16569/1-553
13A	N9a	129 162 223 250 257A 261	73 150 263 309+C 315+C	16018-16569/1-555
13B	N9a	129 162 223 250 257A 261	73 150 263 309+C 315+C	16013-16569/1-553
13C	N9a	129 162 223 250 257A 261	73 150 263 309+C 315+C	16012-16569/1-450
14A	D5	183C 189 362 519	73 150 152 263 309+C 315+C 456 489	16014-16569/1-552
14B	D5	183C 189 362 519	73 150 152 263 309+C 315+C 456 489	16015-16569/1-554
14C	D5	183C 189 362 519	73 150 152 263 309+C 315+C 456 489	16015-16569/1-550
15A	D5	092 148 183C 189 362 519	73 150 152 185 263 315+C 456 489 522-523d	16013-16569/1-552
15B	D5	092 148 183C 189 362 519	73 150 152 185 263 315+C 456 489 522-523d	16013-16569/1-555
15C	D5	092 148 183C 189 362 519	73 150 152 185 263 315+C 456 489 522-523d	16013-16569/1-550
16A	F1	129 172 304 519	73 249d 263 309+2C 315+C 466 520-524d	16007-16569/1-586
16B	F1	129 172 304 519	73 249d 263 **294h** 309+2C 315+C 466 520-524d	16012-16569/1-586
16C	F1	129 172 304 519	73 249d 263 309+2C 315+C 466 520-524d	16016-16569-1-543
17A	M7	129 192 223 297 301G 391 519	73 150 199 263 309+C 315+C 489	16017-16569/1-572
17B	M7	129 192 223 297 301G 391 519	73 150 199 263 309+C 315+C 489	16016-16569/1-544
17C	M7	129 192 223 297 301G 391 519	73 150 199 263 309+C 315+C 489	16020-61569/1-573
18A	A	223 274 290 319 362 519 527	73 152 235 263 315+C 456 522-523d	16015-16569/1-591
18B	A	223 274 290 319 362 519 527	73 152 235 263 315+C 456 522-523d	16009-16569/1-549
18C	A	223 274 290 319 362 519 527	73 152 235 263 315+C 456 522-523d 663 750	16012-16569/1-778
19A	F1a	129 162 172 274 304 519	73 249d 263 315+C 522-523d 548	16016-16569/1-555
19B	F1a	129 162 172 274 304 519	73 249d 263 315+C 522-523d 548	16012-16569/1-620
19C	F1a	129 162 172 274 304 519	73 249d 263 315+C 522-523d 548	16012-16569/1-550
20A	A	223 235 290 311 319 362 519	73 152 234 235 263 **309+2C** 315+C 522-523d	16007-16569/1-555
20B	A	223 235 290 311 319 362 519	73 152 234 235 263 **309+C** 315+C 522-523d 663	16017-16569/1-667
20C	A	223 235 290 311 319 362 519	73 152 234 235 263 **309+2C** 315+C 522-523d	16017-16569/1-575
21A	B4	182C 183C 189 217 223 519	73 146 185 189 195 263 309+2C 315+C	16011-16569/1-322
21B	B4	182C 183C 189 217 223 519	73 146 185 189 195 263 309+2C 315+C 513	16016-16569/1-555

Continued on next page

Table 1. Continued.

Sample	Haplogroups	HVSI (16000+)	HVSII	Readable region
21C	B4	182C 183C 189 217 223 519	73 146 185 189 195 263 309+2C 315+C	16008-16569/1-323
22A	M71	223 269 271 311	73 150 151 263 **309+2C** 315+C 489	16013-16569/1-544
22B	M71	223 269 271 311	73 150 151 263 **309+C** 315+C 489	16012-16569/1-588
22C	M71	223 269 271 311	73 150 151 263 **309+2C** 315+C 489	16010-16569/1-563
23A	F	304	73 249d 263 309+C 315+C	16013-16569/1-563
23B	F	304	73 249d 263 309+C 315+C	16013-16569/1-546
23C	F	304	73 249d 263 309+C 315+C	16009-16569/1-570
24A	C	223 298 327 362 519	73 249d 263 309+C 315+C 489	16010-16569/1-554
24B	C	223 298 327 362 519	73 249d 263 309+C 315+C 489	16009-16569/1-392
24C	C	223 298 327 362 519	73 249d 263 309+C 315+C 489	16008-16569/1-565
25A	H	519	263 309+C 315+C	16012-16569/1-554
25B	H	519	263 **309+2C** 315+C	16012-16569/1-566
25C	H	519	263 309+C 315+C	16012-16569/1-542
26A	A	223 290 319	73 152 235 263 309+2C 315+C 522-523d	16013-16569/1-549
26B	A	223 290 319	73 152 235 263 309+2C 315+C	16011-16569/1-506
26C	A	223 290 319	73 152 235 263 309+2C 315+C 522-523d	16012-16569/1-553
27A	F1a	108 129 162 172 304 519	73 249d 263 315+C 522-523d	16008-16569/1-588
27B	F1a	108 129 162 172 304 519	73 249d 263 315+C 522-523d	16012-16569/1-554
27C	F1a	108 129 162 172 304 519	73 249d 263 315+C 522-523d	16012-16569/1-555
28A	U	051 126 178 179 234 247	73 146 152 263 315+C 522-523d	16009-16569/1-543
28B	U	051 126 178 179 234 247	73 146 152 263 315+C 522-523d	16013-16569/1-556
28C	U	051 126 178 179 234 247	73 146 152 263 315+C 522-523d	16014-16569/1-547
29A	B4a	093 182C 183C 189 217 261 519	73 146 204 263 **309+C** 315+C	16007-16569/1-316
29B	B4a	093 182C 183C 189 217 261 519	73 146 204 263 **309+2C** 315+C 522-523d 709	16013-16569/1-743
29C	B4a	093 182C 183C 189 217 261 519	73 146 204 263 **309+C** 315+C	16013-16569/1-319
30A	G	184 223 290 362 519	73 143 263 315+C 489	16014-16569/1-584
30B	G	184 223 290 362 519	73 143 263 **309+C** 315+C 489	16015-16569/1-590
30C	G	184 223 290 362 519	73 143 263 315+C 489	16132-16569/1-573
31A	F1a	129 172 304 519	73 249d 263 309+2C 315+C 522-523d	16014-16569/1-551
31B	F1a	129 172 304 519	73 249d 263 309+2C 315+C 522-523d	16018-16569/1-584
31C	F1a	129 172 304 519	73 249d 263 309+2C 315+C	16013-16569/1-365
32A	R11	111 172 183C 189 223 362 519	73 185 189 195 234 263 **309+C** 315+C 522-523d	16017-16569/1-550
32B	R11	111 172 183C 189 223 362 519	73 185 189 195 234 263 **309+C** 315+C 522-523d	16016-16569/1-543
32C	R11	111 172 183C 189 223 362 519	73 185 189 195 234 263 **309+3C** 315+C	16012-16569/1-491
33A	A	223 290 319 362	73 151 152 200 235 263 309+C 315+C 522-523d	16012-16569/1-554
33B	A	223 290 319 362	73 151 152 200 235 263 309+C 315+C 522-523d	16013-16569/1-600
33C	A	223 290 319 362	73 151 152 200 235 263 309+C 315+C 522-523d	16013-16569/1-588
34A	D4	124 223 362 519	73 194 263 309+C 315+C 489 522-523d	16016-16569/1-573
34B	D4	124 223 362 519	73 194 263 309+C 315+C	16021-16569/1-414
34C	D4	124 223 362 519	73 194 263 309+C 315+C 489 522-523d	16021-16569/1-572
35A	A	223 290 319 362	73 151 152 200 235 263 315+C 522-523d	16012-16569/1-599
35B	A	223 290 319 362	73 151 152 200 235 263 315+C 522-523d	16015-16569/1-544
35C	A	223 290 319 362	73 151 152 200 235 263 315+C 522-523d	16013-16569/1-587
36A	B4	182C 183C 189 217 261 357 519	73 263 309+C 315+C	16013-16569/1-438
36B	B4	182C 183C 189 217 261 357 519	73 263 309+C 315+C 522-523d	16014-16569/1-586
36C	B4	182C 183C 189 217 261 357 519	73 263 309+C 315+C 522-523d	16017-16569/1-587
37A	D	164 172 182C 183C 189 223 266 362	73 150 263 309+C 315+C 489 522-523d	16017-16569/1-588
37B	D	164 172 182C 183C 189 223 266 362	73 150 263 309+C 315+C 489 522-523d	16013-16569/1-587
37C	D	164 172 182C 183C 189 223 266 362	73 150 263 309+C 315+C 489	16009-16569/1-520
38A	N9a	129 223 257A 261 519	73 150 263 309+C 315+C	16012-16569/1-588
38A	N9a	129 223 257A 261 519	73 150 263 309+C 315+C	16010-16569/1-584
38C	N9a	129 223 257A 261 519	73 150 263 309+C 315+C	16013-16569/1-582
39A	Z	**093h** 185 223 260 271 298 311 390	73 152 249d 263 315+C 319 489	16014-16569/1-588
39B	Z	**093h** 185 223 260 271 298 311 390	73 152 249d 263 315+C 319 489	16014-16569/1-587
39C	Z	**093** 185 223 260 271 298 311 390	73 152 249d 263 315+C 319 489	16014-16569/1-578
40A	A	086 223 290 319 362	73 152 235 263 **309+2C** 315+C 522-523d 538	16012-16569/1-553
40B	A	086 223 290 319 362	73 152 235 263 **309+C** 315+C 522-523d 538	16016-16569/1-555
40C	A	086 223 290 319 362	73 152 235 263 **309+2C** 315+C 522-523d 538	16010-16569/1-585
41A	C	223 298 327 519	73 249d 263 309+C 315+C 489	16013-16569/1-539
41B	C	223 298 327 519	73 249d 263 309+C 315+C 489	16012-16569/1-562
41C	C	223 298 327 519	73 249d 263 309+C 315+C 489	16000-16569/1-573
42A	D/G	223 311 362 519	73 150 194 263 315+C 489 522-523d	16012-16569/1-543

Continued on next page

Table 1. Continued.

Sample	Haplogroups	HVSI (16000+)	HVSII	Readable region
42B	D/G	223 311 362 519	73 150 194 263 315+C 489 522-523d	16013-16569/1-581
42C	D/G	223 311 362 519	73 150 194 263 315+C 489 522-523d	16013-16569/1-588
43A	M7b	129 223 297	73 150 159 199 263 315+C 489	16014-16569/1-559
43B	M7b	129 223 297	73 150 159 199 263 315+C 489	16017-16569/1-587
43C	M7b	129 223 297	73 150 159 199 263 315+C 489	16014-16569/1-550
44A	B5a	140 183C 189 266A 519	73 210 263 **309+2C** 315+C 522-523d	16014-16569/1-554
44B	B5a	140 183C 189 266A 519	73 210 263 **309+C 310d** 315+C 522-523d	16012-16569/1-587
44C	B5a	140 183C 189 266A 519	73 210 263 **309+2C** 315+3C	16018-16569/1-316
45A	F1a	129 172 304 311 519	73 249d 263 315+C 522-523d	16012-16569/1-585
45B	F1a	129 172 304 311 519	73 249d 263 315+C 522-523d	16012-16569/1-587
45C	F1a	129 172 304 311 519	73 249d 263 315+C 522-523d	16013-16569/1-586
46A	B6	093 179 182C 183C 189 342	73 150 263 **309+5C** 522-523d	16016-16569/1-602
46B	B6	093 179 182C 183C 189 342	73 150 263 **309+2C** 522-523d	16016-16569/1-531
46C	B6	093 179 182C 183C 189 342	73 150 263 **309+5C** 522-523d	16013-16569/1-542
47A	M7b	129 192 223 297	73 150 199 263 309+2C 315+C 489 522-523d	16012-16569/1-591
47B	M7b	129 192 223 297	73 150 199 263 309+2C 315+C	16021-16569/1-329
47C	M7b	129 192 223 297	73 150 199 263 309+2C 315+C 489	16021-16569/1-554
48A	A	223 278 290 319 519	73 151 152 235 263 309+C 315+C 522-523d	16013-16569/1-587
48B	A	223 278 290 319 519	73 151 152 235 263 309+C 315+C 522-523d	16013-16569/1-575
48C	A	223 278 290 319 519	73 151 152 235 263 309+C 315+C 522-523d	16013-16569/1-583
49A	B5a	140 183C 189 262 266A 519	73 210 263 315+C 522-523d	16017-16569/1-585
49B	B5a	140 183C 189 262 266A 519	73 210 263 315+C 522-523d	16013-16569/1-588
49C	B5a	140 183C 189 262 266A 519	73 210 263 315+C 522-523d	16009-16569/1-586
50A	F1b	183C 189 323A 249 304 311	73 249d 263 315+C 522-523d	16018-16569/1-586
50B	F1b	183C 189 232A 249 304 311	73 249d 263 315+C 522-523d	16013-16569/1-579
50C	F1b	183C 189 232A 249 304 311	73 249d 263 315+C 522-523d	16013-16569/1-573
51A	D	174 223 311 320 362	73 152 263 309+C 315+C 489	16012-16569/1-586
51B	D	174 223 **293** 311 320 362	73 152 **178** 263 309+C 315+C 489	16009-16569/1-586
51C	D	174 223 311 320 362	73 152 263 309+C 315+C 489	16013-16569/1-587
52A	F	092A 093 234 291 304	73 249d 263 315+C 522-523d	16013-16569/1-586
52B	F	092A 093 234 291 304	73 249d 263 315+C 522-523d	16014-16569/1-546
52C	F	092A 093 234 291 304	73 249d 263 315+C 522-523d	16013-16569/1-546
53A	D4	129 223 278 362	73 263 315+C 489	16013-16569/1-543
53B	D4	129 223 278 362	73 263 315+C 489	16013-16569/1-558
53C	D4	129 223 278 362	73 263 315+C 489	16013-16569/1-554
54A	F1b'd	185 189 266G 291 304 519	73 249d 263 315+C	16017-16569/1-585
54B	F1b'd	185 189 266G 291 304 519	73 249d 263 315+C	16013-16569/1-384
54C	F1b'd	185 189 266G 291 304 519	73 249d 263 315+C	16005-16569/1-588
55A	F1a	108 129 162 172 189 304 519	73 195 245 249d 263 315+C 522-523d	16013-16569/1-587
55B	F1a	108 129 162 172 189 304 519	73 195 245 249d 263 315+C 522-523d 750	16013-16569/1-758
55C	F1a	108 129 162 172 189 304 519	73 195 245 249d 263 315+C 522-523d	16014-16569/1-543
56A	F2	093 203 231 291 **295** 304 519	73 195 249d 263 309+C 315+C	16012-16569/1-554
56B	F2	093 203 231 291 304 519	73 195 249d 263 309+C 315+C	16015-16569/1-591
56C	F2	093 203 231 291 **295** 304 519	73 195 249d 263 309+C 315+C	16000-16569/1-620
57A	A	223 260 290 319 519	64 73 146 195 235 263 309+C 315+C 522-523d	16021-16569/1-533
57B	A	223 260 290 319 519	64 73 146 195 235 263 309+ C 315+C 522-523d 663 750	16014-16569/1-777
57C	A	223 260 290 319 519	64 73 146 195 235 263 309+2C 315+C 522-523d	16017-16569/1-541
58A	B5a	093 140 183C 189 266A 519	73 146 198 210 263 309+2C 315+C 522-523d	16016-16569/1-543
58B	B5a	093 140 183C 189 266A 519	73 146 198 210 263 309+2C 315+C 522-523d	16012-16569/1-542
58C	B5a	093 140 183C 189 266A 519	73 146 198 210 263 309+2C 315+C 522-523d	16013-16569/1-555
59A	M9a1a	223 234 248 265C 316 362	73 153 263 309+C 315+C 489	16014-16569/1-573
59B	M9a1a	223 234 248 265C 316 362	73 153 263 309+C 315+C 489	16009-16569/1-543
59C	M9a1a	223 234 248 265C 316 362	73 153 263 309+C 315+C 489	16011-16569/1-543
60A	C	223 290 298 327 519	73 146 152 249d 263 315+C 489	16013-16569/1-584
60B	C	223 290 298 327 519	73 146 152 249d 263 315+C 489	16014-16569/1-584
60C	C	223 290 298 327 519	73 146 152 249d 263 315+C 489	16013-16569/1-585
61A	N9a	189 223 257A 261 311	73 150 263 309+2C 315+C	16016-16569/1-579
61B	N9a	189 223 257A 261 311	73 150 263 309+2C 315+C	16015-16569/1-582
61C	N9a	189 223 257A 261 311	73 150 263 309+2C 315+C	16014-16569/1-572
62A	B5b2	093 111 140 182C 183C 189 234 243 463 519	73 103 131 146 199 204 263 **309+3C** 315+C	16017-16569/1-323
62B	B5b2	093 111 140 182C 183C 189 234 243 463 519	73 103 131 146 199 204 263 **309+2C** 315+C	16012-16569/1-317

Continued on next page

Table 1. Continued.

Sample	Haplogroups	HVSI (16000+)	HVSII	Readable region
62C	B5b2	093 111 140 182C 183C 189 234 243 463 519	73 103 131 146 199 204 263 **309+3C** 315+C	16011-16569/1-322
63A	B4a	182C 183C 186 189 217 261 360 519	73 263 315+C 522-523d	16013-16569/1-583
63B	B4a	182C 183C 186 189 217 261 360 519	73 263 315+C 522-523d	16009-16569/1-565
63C	B4a	182C 183C 186 189 217 261 360 519	73 263 315+C 522-523d	16009-16569/1-580
64A	D4	129 223 278 362	73 263 315+C 489	16013-16569/1-580
64B	D4	129 223 278 362	73 263 315+C 489	16011-16569/1-585
64C	D4	129 223 278 362	73 263 315+C 489	16011-16569/1-565
65A	D4b	287 319 362 390	73 263 315+C **420h** 431 489 522-523d	16007-16569/1-587
65B	D4b	287 319 362 390	73 263 315+C **420** 431 489 522-523d	16016-16569/1-585
65C	D4b	287 319 362 390	73 263 315+C **420h** 431 489 522-523d	16013-16569/1-584
66A	B4	182C 183C 189 217 240 261	73 263 309+C 315+2C 522-523d	16013-16569/1-554
66B	B4	182C 183C 189 217 240 261	73 263 309+2C 315+C 522-523d	16017-16569/1-553
66C	B4	182C 183C 189 217 240 261	73 263 309+2C 315+C 522-523d	16016-16569/1-543
67A	Z	172 185 **189** 223 260 298 **362** 519	73 151 152 249d 263 315+C 489	16014-16569/1-587
67B	Z	172 185 223 260 298 519	73 151 152 249d 263 315+C 489	16013-16569/1-583
67C	Z	172 185 **189** 223 260 298 **362** 519	73 151 152 249d 263 315+C 489	16013-16569/1-585
68A	D4	223 256 311 362 519	73 200 263 309+C 315+C 489 522-523d	16021-16569/1-577
68B	D4	223 256 311 362 519	73 200 263 309+C 315+C 489 522-523d	16013-16569/1-584
68C	D4	223 256 311 362 519	73 200 263 309+C 315+C 489 522-523d	16013-16569/1-585
69A	F2	124 167 203 304 318 519	73 249d 263 315+C	16013-16569/1-560
69B	F2	124 167 203 304 318 519	73 249d 263 315+C	16016-16569/1-585
69C	F2	124 167 203 304 318 519	73 249d 263 315+C	16009-16569/1-573
70A	B5a	140 187 189 256 266G 519	73 93 210 263 315+C 522-523d	16009-16569/1-585
70B	B5a	140 187 189 256 266G 519	73 93 210 263 315+C 522-523d	16013-16569/1-585
70C	B5a	140 187 189 256 266G 519	73 93 210 263 315+C 522-523d	16008-16569/1-571
71A	D4	129 223 278 362	73 263 315+2C 489 523+CA	16013-16569/1-585
71B	D4	129 223 278 362	73 263 315+2C 489 523+CA	16012-16569/1-585
71C	D4	129 223 278 362	73 263 315+2C 489 523+CA	16013-16569/1-562
72A	B4a	182C 183C 189 217 261	73 200 263 **309+2C** 522-523d	16016-16569/1-543
72B	B4a	182C 183C 189 217 261	73 200 263 **309+CCCCA** 522-523d	16016-16569/1-543
72C	B4a	182C 183C 189 217 261	73 200 263 **309+2C** 522-523d	16008-16569/1-554
73A	Z	185 223 260 297 298	73 152 189 207 249d 263 309+2C 315+C 489	16006-16569/1-567
73B	Z	185 223 260 297 298	73 152 189 207 249d 263 309+2C 315+C 489	16012-16569/1-543
73C	Z	185 223 260 297 298	73 152 189 207 249d 263 309+2C 315+C 489	16012-16569/1-520
74A	M31	093 136 223	73 146 152 263 315+C 489	16010-16569/1-567
74B	M31	093 136 223	73 146 152 263 315+C 489	16006-16569/1-569
74C	M31	093 136 223	73 146 152 263 315+C 489	16024-16569/1-588
75A	B5b2	093 182C 183C 189 217 243 261 519	73 146 204 263 **309+C** 315+C 522-523d	16014-16569/1-587
75B	B5b2	093 182C 183C 189 217 243 261 519	73 146 204 263 **309+C** 315+C 522-523d	16013-16569/1-543
75C	B5b2	093 182C 183C 189 217 243 261 519	73 146 204 263 **309+2C** 315+C	16012-16569/1-316
76A	D5	189 223 362 519	73 150 263 309+2C 315+C 456 489 523+CA	16017-16569/1-543
76B	D5	189 223 362 519	73 150 263 309+2C 315+C 456 489 523+CA	16012-16569/1-543
76C	D5	189 223 362 519	73 150 263 309+2C 315+C	16010-16569/1-316
77A	B4a	093 182C 183C 189 217 261 519	73 146 263 309+C 315+C	16012-16569/1-318
77B	B4a	093 182C 183C 189 217 261 519	73 146 263 309+C 315+C 522-523d	16012-16569/1-554
77C	B4a	093 182C 183C 189 217 261 519	73 146 263 309+C 315+C 522-523d	16012-16569/1-550
78A	R9	304 362 519	73 263 315+C	16007-16569/1-590
78B	R9	304 362 519	73 263 315+C	16007-16569/1-587
78C	R9	304 362 519	73 263 315+C	16015-16569/1-368
79A	B4a	140 183C 189 266A 311 519	73 210 263 315+C 522-523d	16008-16569/1-558
79B	B4a	140 183C 189 266A 311 519	73 210 263 315+C 522-523d	16010-16569/1-550
79C	B4a	140 183C 189 266A 311 519	73 210 263 315+C 522-523d	16009-16569/1-542

As shown in Table 2, the frequencies of mtDNA haplogroups F* and G* showed significant differences between the lung cancer and control group, which indicated the possible associations between mtDNA haplogroups F* and G* and lung cancer groups from Yunnan, Southwest of China (at the level of $P < 0.05$).

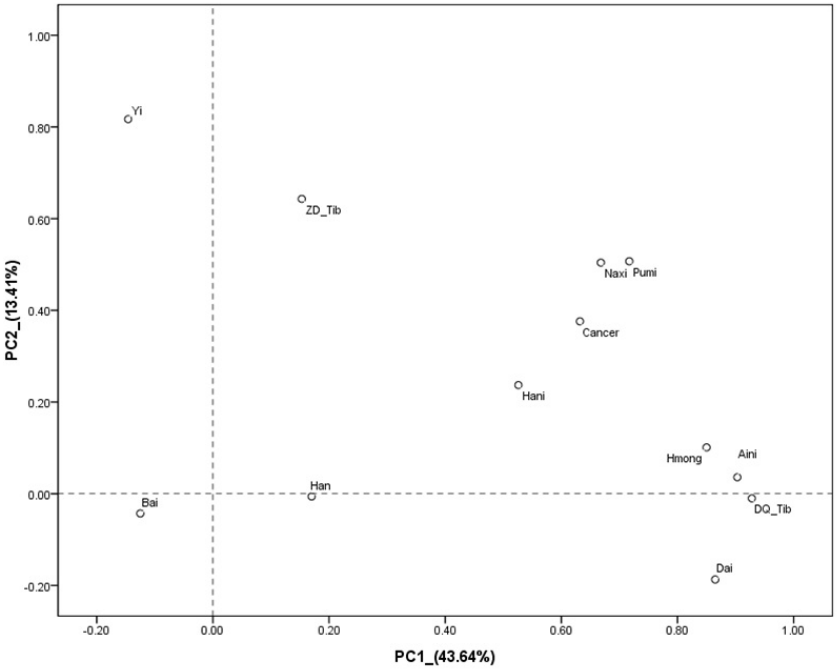

Figure 1. Principle component analysis (PCA) of the populations under study. ZD_Tib = Tibetan group from Zhongdian region of Yunnan Province; DQ_Tib = Tibetan group from Diqing region of Yunnan Province.

Table 2. Haplogroup frequent and Pearson chi-square test results in patients with lung cancer and controls from Yunnan, Southwest China.

Haplogroups	Lung cancer (N)	Control (N)	χ^2	P value	OR	95%CI
A	8	34	1.011	0.315	1.151	0.672-3.396
B	1	5	0.039	0.594	1.244	0.143-10.787
B4	10	47	0.71	0.4	1.366	0.660-2.829
B5	6	27	0.541	0.439	1.409	0.563-3.351
C	3	27	0.4	0.786	0.677	0.200-2.286
D	4	33	0.313	0.576	0.739	0.254-2.145
D4	9	49	0.144	0.704	1.157	0.544-2.460
D5	3	19	0.001	1	0.979	0.283-3.387
F	3	3	6.651	0.038	6.408	1.270-32.330
F1	10	51	0.36	0.549	1.248	0.605-2.573
F2	2	12	0.002	1	1.035	0.227-4.712
G	1	37	4.312	0.038	0.157	0.021-1.161
M	3	14	0.208	0.718	1.342	0.377-40478
M7	4	35	0.461	0.497	0.693	0.240-2.007
M9	1	18	1.222	0.496	0.336	0.044-2.554
N9	3	4	4.976	0.06	4.976	1.053-21.849
R	2	2	4.395	0.095	6.338	0.880-45.657
R11	1	5	0.039	0.054	1.244	0.143-10.787
R9	2	7	0.532	0.362	1.792	0.366-8.786
Z	3	6	2.893	0.116	3.184	0.780-13.001

*P value was calculated by the Pearson chi-square test at the level of $P < 0.05$; the Fisher exact test was used when haplogroups were expected to contain fewer than five individuals.

Furthermore, as shown in our previous study, a large number of the identified somatic mutations among the patients with lung cancer from Yunnan, Southwest China, were detected by sequencing the entire mtDNA genome (Fang et al., 2013) and the mtDNA D310 region (Chen et al., 2014). In view of the relatively higher somatic mutation rate in the whole mtDNA D-loop (Yu 2012), and the relatively small sample sizes (Fang et al., 2013) and limited information obtained from our previous studies, which only analyzed the sequence variations within the mtDNA D310 region (Chen et al., 2014), we therefore sequenced and analyzed the complete mtDNA control region (except for the polymorphisms at D310) of 79 patients with lung cancer from Yunnan, Southwest China. Somatic mutations were detected by comparison of the sequences between tumor, matched normal tissues, and blood from the same patients, with the aim to provide more information toward better understanding the potential role of a given somatic mutation in tumorigenesis. As listed in Table 1, a total of eight somatic mutations were detected among six patients with lung cancer, including mutations at positions 16,093, 16,189, 16,362, 16,293, and 16,295 at HVSI, and mutations 178, 294 and 420 at HVSII/III. As shown in Figure 2, the authenticity of the eight somatic mutations was verified using the strategy described in our previous study (Fang et al., 2013; Chen et al., 2014). Incorporating the polymorphisms at D310 as reported in our previous study (Chen et al., 2014), a total of 20 individuals with somatic mutations were detected among 79 patients with lung cancer from Yunnan, Southwest China, which accounted for 24.05% (19/79) of the patients with lung cancer examined. By further analyzing these somatic mutations, we found that heterogeneity was the predominant characteristic of somatic mutations, in line with our previous results (Fang et al., 2013; Chen et al., 2014).

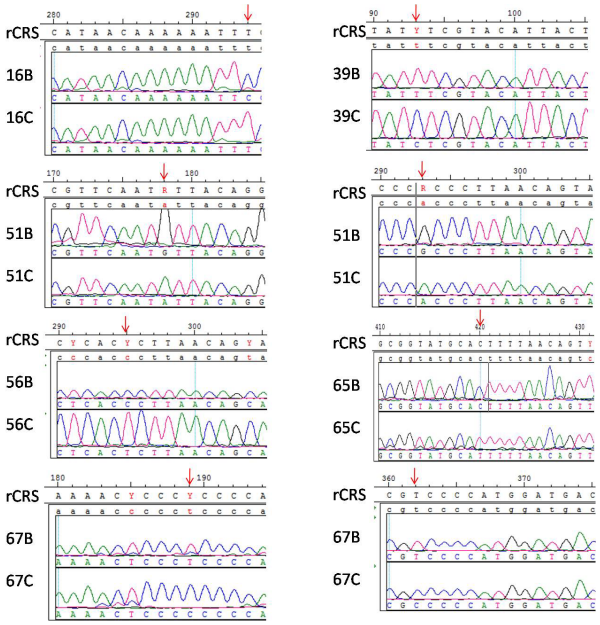

Figure 2. Somatic mtDNA mutations detected at the mtDNA control region in 79 Chinese patients with lung cancer. Sequencing results of the lung cancer tissue and distant normal tissue from the same patient are shown; sites of mutation are marked with red arrows. Mutations were identified by comparison with the revised Cambridge reference sequence (rCRS). B: mtDNA sequence from the primary tumor sample; C: mtDNA sequence from peripheral blood from the same patient.

DISCUSSION

By extensively analyzing the mtDNA variations in 237 samples from 79 patients with lung cancer from Yunnan, we found that the frequency of East Asian and Southeast Asian prevalent haplogroups (50.63%, 40/79) was higher than that of northern East Asian prevalent haplogroups (44.30%, 35/79) of the patients. These haplogroup distributions were in line with those of healthy control groups from Yunnan Province (Yao et al., 2002b; Wen et al., 2004b), which may be a result of the admixture of autochthonous components from both northern and southern populations.

A total of 20 somatic mutations were detected among 19 of 79 patients with lung cancer from Yunnan, which implied the mtDNA somatic mutations confer genetic susceptibility to lung cancer in patients from Yunnan, Southwest China, and heterogeneity was the major characteristic of the 20 somatic mutations. The mtDNA D-loop is important for regulation of mitochondrial genome replication and expression. Therefore, the relatively elevated somatic mutation rates in the patients with lung cancer from Yunnan Province, Southwest China, suggested that the somatic mutations in these patients might affect the crucial mitochondrial function in the electron transport chain, which might in turn cause a high release of reactive oxygen species and concomitant nuclear genome damage as well as cancer initiation and promotion (Shigenaga et al., 1994; Li et al., 2012; Yu, 2012).

In summary, to test whether maternal background or mtDNA somatic mutations played a crucial role in Chinese patients with lung cancer, both the germline and somatic mutations of the mtDNA D-loop region were analyzed in 237 samples from 79 Chinese patients with lung cancer. The PCA and statistical analysis rejected the likelihood of population stratification, and further statistical analysis supported the existence of associations between mtDNA haplogroups F* and G* and the Chinese lung cancer group. Furthermore, a higher frequency of somatic mutations (25.32%) in the mtDNA D-loop was detected among Chinese patients with lung cancer, which indicated that the somatic mutations might play crucial roles in the initiation and promotion of lung cancer. Our results suggest that mitochondrial DNA haplogroups and somatic mutations confer genetic susceptibility to lung cancer in patients from Southwest China, and that the somatic mtDNA mutations in the D-loop can serve as a potential biomarker for clinical utility.

ACKNOWLEDGMENTS

We are grateful to all the voluntary donors for participating in the project. Research supported by grants from the Nature Sciences Foundation of Yunnan Provincial Science and Technology Department (#2012CA002 and #2012HB030).

REFERENCES

Alhomidi MA, Vedicherla B, Movva S, Rao PK, et al. (2013). Mitochondrial D310 instability in Asian Indian breast cancer patients. *Tumour Biol.* 34: 2427-2432.

Anderson S, Bankier AT, Barrell BG, Bruijn MH, et al. (1981). Sequence and organization of the human mitochondrial genome. *Nature* 290: 457-465.

Andrews RM, Kubacka I, Chinnery PF, Lightowlers RN, et al. (1999). Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. *Nat. Genet*. 23: 147.

Biffi A, Anderson CD, Nalls MA, Rahman R, et al. (2010). Principal-component analysis for assessment of population stratification in mitochondrial medical genetics. *Am. J. Hum. Genet*. 86: 904-917.

Brandon M, BaldiP and Wallace DC (2006). Mitochondrial mutations in cancer. *Oncogene* 25: 4647-4662.

Cao C, Zhang YM, Wang R, Sun SF, et al. (2011). Excision repair cross complementation group 1 polymorphisms and lung cancer risk: a meta-analysis. *Chin. Med. J*. 124: 2203-2208.

Chatterjee A, Mambo E and Sidransky D (2006). Mitochondrial DNA mutations in human cancer. *Oncogene* 25: 4663-4674.

Chen XZ, Fang Y, Shi YH, Cui JH, et al. (2014). Mitochondrial D310 instability in Chinese lung cancer patients. *Mitochondrial DNA* 10: 1-4. Doi: 10.3109/19401736.19402014.19936426.

Fang Y, Huang J, Zhang J, Wang J, et al. (2013). Detecting the somatic mutations spectrum of Chinese lung cancer by analyzing the whole mitochondrial DNA genomes. *Mitochondrial DNA* Sept. 6. [Epub ahead of print]. Doi: 10.3109/19401736.2013.823168.

Ferlay J, Shin HR, Bray F, Forman D, et al. (2010). Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int. J. Cancer* 127: 2893-2917.

Fuku N, Park KS, Yamada Y, Nishigaki Y, et al. (2007). Mitochondrial haplogroup N9a confers resistance against type 2 diabetes in Asians. *Am. J. Hum. Genet*. 80: 407-415.

Hung WY, Wu CW, Yin PH, Chang CJ, et al. (2010). Somatic mutations in mitochondrial genome and their potential roles in the progression of human gastric cancer. *Biochim. Biophys. Acta* 1800: 264-270.

Ji Y, Zhang AM, Jia XY, Zhang YP, et al. (2008). 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: 760-768.

Jin X, Zhang J, Gao Y, Ding K, et al. (2007). Relationship between mitochondrial DNA mutations and clinical characteristics in human lung cancer. *Mitochondrion* 7: 347-353.

Kong QP, Sun C, Wang HW, Zhao M, et al. (2011). Large-scale mtDNA screening reveals a surprising matrilineal complexity in East Asia and its implications to the peopling of the region. *Mol. Biol. Evol.* 28: 513-522.

Kumimoto H, Yamane Y, Nishimoto Y, Fukami H, et al. (2004). Frequent somatic mutations of mitochondrial DNA in esophageal squamous cell carcinoma. *Int. J. Cancer* 108: 228-231.

Li H, Liu D, Lu J and Bai Y (2012). Physiology and pathophysiology of mitochondrial DNA. *Adv. Exp. Med. Biol*. 942: 39-51.

Mishmar D, Ruiz-Pesini E, Golik P, Macaulay V, et al. (2003). Natural selection shaped regional mtDNA variation in humans. *Proc. Natl. Acad. Sci. U. S. A*. 100: 171-176.

Ruiz-Pesini E, Mishmar D, Brandon M, Procaccio V, et al. (2004). Effects of purifying and adaptive selection on regional variation in human mtDNA. *Science* 303: 223-226.

Sharma H, Singh A, Sharma C, Jain SK, et al. (2005). Mutations in the mitochondrial DNA D-loop region are frequent in cervical cancer. *Cancer Cell Int*. 5: 34.

Shigenaga MK, Hagen TM and Ames BN (1994). Oxidative damage and mitochondrial decay in aging. *Proc. Natl. Acad. Sci. U. S. A*. 91: 10771-10778.

van Oven M and Kayser M (2009). Updated comprehensive phylogenetic tree of global human mitochondrial DNA variation. *Hum. Mutat.* 30: e386-394.

Wallace DC (1995). Mitochondrial DNA variation in human evolution, degenerative disease, andaging. *Am. J. Hum. Genet*. 57: 201-223.

Wallace DC (2005). A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. *Annu. Rev. Genet*. 39: 359-407.

Wallace DC, Brown MD and Lott MT (1999). Mitochondrial DNA variation in human evolution and disease. *Gene* 238: 211-230.

Wang CY and Zhao ZB (2011). Somatic mtDNA mutations in lung tissues of pesticide-exposed fruitgrowers. *Toxicology* 291: 51-55.

Wang CY, Wang HW, Yao YG, Kong QP, et al. (2007). Somatic mutations of mitochondrial genome in early stage breast cancer. *Int. J. Cancer* 121: 1253-1256.

Wen B, Li H, Lu D, Song X, et al. (2004a). Genetic evidence supports demic diffusion of Han culture. *Nature* 431: 302-305.

Wen B, Xie X, Gao S, Li H, et al. (2004b). Analyses of genetic structure of Tibeto-Burman populations reveals sex-biased admixture in southern Tibeto-Burmans. *Am. J. Hum. Genet.* 74: 856-865.

Wen B, Li H, Gao S, Mao X, et al. (2005). Genetic structure of Hmong-Mien speaking populations in East Asia as revealed by mtDNA lineages. *Mol. Biol. Evol.* 22: 725-734.

Williams SL, Mash DC, Zuchne S and Moraes CT (2013). Somatic mtDNA mutation spectra in the aging human putamen. *PLoS Genet.* 9: e1003990.

Yakes FM and Van Houten B (1997). Mitochondrial DNA damage is more extensive and persistslonger than nuclear DNA damage in human cells following oxidative stress. *Proc. Natl. Acad. Sci. U. S. A.* 94: 514-519.

Yang L, Yang G, Zhou M, Smith M, et al. (2009). Body mass index and mortality from lung cancer in smokers and nonsmokers: a nationally representative prospective study of 220,000 men in China. *Int. J. Cancer* 125: 2136-2143.

Yang Ai SS, Hsu K, Herbert C, Cheng Z, et al. (2013). Mitochondrial DNA mutations in exhaled breath condensate of patients with lung cancer. *Respir. Med.* 107: 911-918.

Yao YG, Kong QP, Bandelt HJ, Kivisild T, et al. (2002a). Phylogeographic differentiation of mitochondrial DNA in Han Chinese. *Am. J. Hum. Genet.* 70: 635-651.

Yao YG, Nie L, Harpending H, Fu YX, et al. (2002b). Genetic relationship of Chinese ethnic populations revealed by mtDNA sequence diversity. *Am. J. Phys. Anthropol*. 118: 63-76.

Yu M (2012). Somatic mitochondrial DNA mutations in human cancers. *Adv. Clin. Chem.* 57: 99-138.

Zhao M, Kong QP, Wang HW, Peng MS, et al. (2009). Mitochondrial genome evidence reveals successful Late Paleolithic settlement on the Tibetan Plateau. *Proc. Natl. Acad. Sci. U. S. A*. 106: 21230-21235.