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Revisiting the matrilineal lineages and hypoxic adaptation of highland Tibetans

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ABSTRACT

The mitochondrial DNA (mtDNA) plays a vital role in forensic, anthropological, biogeographical and genealogical studies. In the present study, we sequenced 59 mitochondrial genomes of Tibetan individuals settling in Muli Tibetan Autonomous County of Sichuan Province using the Precision ID mtDNA Whole Genome Panel and Ion S5 XL system. Meanwhile, 192 published complete mitogenomes from five Tibetan populations were included for further analysis. All 251 investigated Tibetan participants were assigned to 98 unique subclades pertained to the macrohaplogroups M and N, and 17 subhaplogroups were considered as major haplogroups of Tibetans since these subhaplogroups accounted for considerably high frequencies in randomly selected Tibetans. It was noteworthy that M9a1a1c1b1a was the predominant subhaplogroup in the Tibetans collectively. Furthermore, the nonsynonymous substitutions and synonymous substitutions ratios (N/S) of Tibetans, Tibetan highlanders (Monpas, Lhobas, Dengs and Sherpas), non-Tibetan highlanders and general populations were estimated to evaluate the potential selective constraints. The N/S ratio in the Tibetan groups (0.503) is higher than that in Tibetan highlanders (0.465), non-Tibetan highlanders (0.430) and general populations (0.415). The distributions of N/S ratio in 13 protein-coding genes revealed that significant differences were existed in COX2, ATP8 genes, which likely contributed to hypoxic adaptation.

1. Introduction

Tibetan people, mainly residing in Tibetan Plateau areas of China, is one of the oldest ethnic groups in China and South Asia. As of the 2016 census, the current population of Tibetan nationality is estimated approximately 7 million within China. Tibetans speak Tibetic languages, which belongs to the Tibeto-Burman languages of the Sino-Tibetan language families. Tibetans arose from a mixture of multiple ancestral gene pools, the origin and demographic history of Tibetans remain one of the most controversial puzzles in anthropology, archaeology, genetics and forensics [1]. Present-day Tibetans, whose ancestors began to colonize the Tibetan Plateau before the Last Glacial Maximum (LGM), had the highest rate of allele sharing with archaic hominins (Denisovans, Neanderthals, ancient-Siberians and unknown ancestries). Besides, previous genetic studies demonstrated that the majority of the Tibetan gene pool has derived from modern human and diverged from Han Chinese around 9000 to 15,000 years ago [1]. Tibetans exhibit high-altitude adaptations, which has been attributed to mutations in the EPAS1 gene introgressed from Denisovans. The uniparentally inherited mitochondrial DNA (mtDNA), an extra-nuclear circular molecule with high mutation rate, offers the potential to uncover maternal evolutionary history. Mitochondrial genome (mitogenome) encoding 13 core subunits of oxidative phosphorylation (OXPHOS) plays a vital role in metabolism and is regarded as a reasonable object for studying hypoxic adaptation in Tibetans [2,3]. Here, we sequenced the whole mitogenome sequences of 59 Tibetans residing in Muli Tibetan Autonomous County of Sichuan Province by massively parallel sequencing (MPS) technology, which provides a broader conduit to reconstruct the demographic history of Tibetans and investigate the effects of natural selection on the characteristic variants of mitogenome in Tibetans.

2. Materials and methods

2.1. Sample collection and sequencing

Peripheral-blood samples of 59 Tibetans were collected from Muli Tibetan Autonomous County in the Liangshan Yi Autonomous Prefecture, Sichuan Province. This study strictly followed the recommendations of the Declaration of Helsinki [4] and was approved by the Ethics Committee at the Institution of Forensic Medicine, Sichuan University. Genomic DNA (gDNA) was extracted using the QIAamp DNA Mini Kit (Qiagen) and quantified with the Quantifiler Human DNA

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Table 1
The distribution of nonsynonymous/synonymous mutations in mtDNA protein-coding regions.

Gene	Tibetans			Tibetan Highlanders				Non-Tibetan Highlanders				General populations			
	N	S	N/S	N	S	N/S	P	N	S	N/S	P	N	S	N/S	P
ATP6	30	20	1.500	24	17	1.412	0.888	22	16	1.375	0.842	31	32	0.969	0.253
ATP8	10	7	1.429	7	3	2.333	0.692	4	5	0.800	0.683	7	12	0.583	0.316
COX1	9	53	0.170	6	43	0.140	0.728	7	45	0.156	0.872	14	81	0.173	0.970
COX2	10	26	0.385	11	20	0.550	0.498	2	27	0.074	0.031	11	40	0.275	0.505
COX3	9	25	0.360	2	26	0.077	0.047	12	26	0.462	0.634	14	45	0.311	0.768
CYTB	29	46	0.630	25	31	0.806	0.492	32	36	0.889	0.311	51	74	0.689	0.766
ND1	20	39	0.513	16	25	0.640	0.599	19	32	0.594	0.714	26	51	0.510	0.987
ND2	19	40	0.475	15	26	0.577	0.649	15	39	0.385	0.608	28	55	0.509	0.848
ND3	6	13	0.462	1	14	0.071	0.104	3	14	0.214	0.451	8	20	0.400	0.825
ND4L	2	10	0.200	2	5	0.400	0.603	3	8	0.375	0.64	4	15	0.267	1.000
ND4	9	37	0.243	8	34	0.235	0.951	9	45	0.200	0.707	19	65	0.292	0.685
ND5	34	57	0.596	22	55	0.400	0.228	26	59	0.441	0.343	35	103	0.340	0.053
ND6	8	15	0.533	7	15	0.467	0.833	6	20	0.300	0.365	9	27	0.333	0.418
TOTAL	**195**	**388**	**0.503**	**146**	**314**	**0.465**	**0.559**	**160**	**372**	**0.430**	**0.227**	**257**	**620**	**0.415**	**0.094**

Quantification Kit (Thermo Fisher Scientific) on an Applied Biosystem 7500 Real-time PCR System (Thermo Fisher Scientific) following the manufacturer's instructions. DNA samples were stored at −20 °C until amplification. Library preparation was performed with the Precision ID Library Kit and the Precision ID mtDNA Whole Genome Panel (Thermo Fisher Scientific) using "conservative" method according to the manufacturer's protocol. Each library was purified with AMPure XP reagent (Beckman Coulter) and quantified using the Ion Library TaqMan Quantitation kit (Thermo Fisher Scientific). To balance the sample input concentration, the samples were diluted to a concentration of 30 pM. Subsequently, template preparation was carried out automatically using the Ion 520 & Ion 530 Kit and Ion 530 chips with the Ion Chef System (Thermo Fisher Scientific). Sequencing was performed on the Ion S5 XL Sequencer (Thermo Fisher Scientific) following the manufacturer's instructions.

2.2. Data processing

All mitogenome sequences were mapped to the revised Cambridge Reference Sequence (rCRS + 80) and analyzed using the Torrent Suite Software v5.6.0 (Thermo Fisher Scientific). The plugins Variant Caller v5.6.0.4, Coverage Analysis v5.6.0.1 and Integrative Genomic Viewer (IGV) v2.3.97 [5] were introduced for secondary analysis following the International Society for Forensic Genetics (ISFG) guidelines [6]. Haplogroup assignment was performed using HaploGrep 2 based on PhyloTree build 17 [7]. Subsequently, complete mitogenomes of 192 Tibetans, 294 Tibetan highlanders (Monpas, Lhobas, Dengs and Sherpas), 270 non-Tibetan highlanders and 320 Hans were retrieved for comparisons after stringent quality-control procedure. The N/S ratios of four population datasets (Tibetan groups, Tibetan highlanders, non-Tibetan highlanders and general populations) were estimated to evaluate the potential selective constraints by dividing the number of nonsynonymous (N) substitutions by the number of synonymous (S) substitutions in the mtDNA protein-coding regions. Fisher's exact test was utilized to assess the differences of N/S ratio, and the $P < 0.05$ was considered statistically significant.

3. Results and discussion

All Tibetan mitogenomes investigated in this study were assigned to 98 unique subclades and consisted of major East Asian haplogroups M9, G, A, R9 and D. 17 subhaplogroups were considered as major haplogroups of Tibetans, and M9a1a1c1b1a (12.35%) was regarded as the most frequent subhaplogroup in the Tibetans collectively. The N/S value in the Tibetan populations (0.503) is higher than that in Tibetan highlanders (0.465), non-Tibetan highlanders (0.430) and general populations (0.415) (Table 1). In the Tibetan groups, the N/S values of ATP6 and ATP8 genes are > 1, demonstrating possible adaptive selection, whereas other genes might pertain to purifying selection (N/S < 1). Furthermore, we found that the distributions of N/S ratio in 13 protein-coding genes showed no significant differences between Tibetans and other three population sets except for COX3 and COX2 genes. In COX3 gene, the N/S ratio in the Tibetans is significantly higher ($P = 0.047$) than that in the Tibetan highlanders, and in COX2 gene, the N/S ratio in the Tibetans is significantly higher ($P = 0.031$) than that in the non-Tibetan highlanders, demonstrating that some of the nonsynonymous substitutions might have been favorably preserved in Tibetans, which was a signal of positive selection.

4. Conclusion

The mitogenome sequences generated in present study have enriched existing mtDNA database and provided resources for dissecting population stratification of worldwide populations. The comprehensive haplogroup analysis of Tibetan populations has shed light on the genetic substructure of different Tibetan groups. The comparison of N/S ratios provided evidence suggestive of adaptive selection for ATP6, ATP8, COX3 and COX2. Further studies are necessary to reveal the mechanism how mtDNA mutations contribute to hypoxia adaptation.

Declaration of Competing Interest

The authors declare that they have no conflicts of interest.

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References

[1] D. Lu, H. Lou, K. Yuan, et al., Ancestral origins and genetic history of Tibetan highlanders, Am. J. Hum. Genet. 99 (2016) 580–594.
[2] M. Gu, X. Dong, L. Shi, et al., Differences in mtDNA whole sequence between Tibetan and Han populations suggesting adaptive selection to high altitude, Gene 496 (2012) 37–44.
[3] Q. Li, K. Lin, H. Sun, et al., Mitochondrial haplogroup M9a1a1c1b is associated with hypoxic adaptation in the Tibetans, J. Hum. Genet. 61 (2016) 1021–1026.
[4] A. Nicogossian, O. Kloiber, B. Stabile, The revised world medical association's declaration of Helsinki 2013: enhancing the protection of human research subjects and empowering ethics review committees, World Med. Health Policy 6 (2014) 1–3.
[5] H. Thorvaldsdottir, J.T. Robinson, J.P. Mesirov, Integrative genomics viewer (IGV): high-performance genomics data visualization and exploration, Brief Bioinform. 14

(2013) 178–192.
[6] W. Parson, L. Gusmao, D.R. Hares, et al., DNA commission of the international society for forensic genetics: revised and extended guidelines for mitochondrial DNA typing, Forensic Sci. Int. Genet. 13 (2014) 134–142.
[7] M. van Oven, M. Kayser, Updated comprehensive phylogenetic tree of global human mitochondrial DNA variation, Hum. Mutat. 30 (2009) E386–394.