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MITOCHONDRIAL DNA EVIDENCE OF SOUTHWARD MIGRATION OF MANCHUS IN CHINA

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The Northeast area of China is a cross region between East Asia and Siberia. Although five populations from this area have been studied in maternal lineage, little is known about the genetics of other populations. In this study, forty-seven Manchu individuals were analyzed using a mitochondrial DNA marker, and fourteen mitochondrial DNA haplogroups, the representative haplogroups of east Eurasian, were identified. All analyses showed that Manchu were close to the neighboring populations such as Mongolian, Korean and northern Han Chinese, and were far from the other populations who lived in the cradle of Manchu, suggesting that the Manchu integrated gradually with natives following its southward migration.

***Keywords*: Manchu, mitochondrial DNA, North Asia, northeastern China.**

INTRODUCTION

Recently, many Chinese ethnic groups have been analyzed to uncover their genetic structures using different genetic markers, especially the widely adopted mitochondrial DNA (MtDNA) marker [1–6], which can better trace the migration and expansion of the Chinese people. Analysis of the populations from northeastern China, a cross region between East Asia and Siberia, will uncover the genetic exchange between North Asians and East Asians, and will help to better understand the migration pattern of East Asians. Nevertheless, there is still a fact that the populations from southern China were much more sampled than those from northern China. Only five ethnic populations from the northeastern China had hitherto been analyzed in maternal lineage [6]. The Manchu people from northeastern China, who played a very important role in Chinese history, have not been considered. According to historical documents, the Manchus traced their origin to the populations who lived around the Amur-Heilong River valley. They began to migrate southward across the whole Northeastern China about 650 years ago. After the Qing Empire was built, a large number of Manchu people moved into the area where the Han Chinese lived, and many of them mixed gradually with the Han Chinese population [7]. In this study, we examined mtDNA extracted from 47 Manchus and compared the data with those of relevant populations.

EXPERIMENTAL

A total of 47 individuals from the Manchu population residing in different towns and villages in the Jilin province, China (fig. 1), were sampled and were confirmed to be unrelated. All of the individuals gave informed consent. Fragments (16050–16409) of the mitochondrial hypervariable region I (HVR-I) were amplified using the primer pair L16017 (5'- TTCTCTGTTCTT-TCATGGGGA-3') / H16409 (5'-AGGATGGTGGT-CAAGGGA-3'). The mitochondrial haplogroups were assigned using the data on SNPs in the hypervariable region and the coding region. Haplogroups M(10400T), A(663G), C(14318C), D (5178A), D4(3010A), F (3970T), G(4833G), N9a(5417A) and Z(152C) were examined using the amplified product-length polymorphism method [8]. Haplogroup B was checked by screening for the 9 bp deletion in the COII/tRNALys region. Haplogroups D5 and F1b were identified by sequencing position 16189 in advance, and haplogroups F1a and F1c by sequencing position 16172 and 16111 respectively. Haplogroup M8a was checked by sequencing positions 16298 and 16319, and haplogroup M7 by sequencing positions 16223, 16260 and 16297. The sequences were edited and aligned by DNAstar software and compared with the revised Cambridge reference sequence [9]. The data on the northern minorities of China [6], northern Asians [10, 11], Outer Mongolians [11], Koreans [11], Han Chinese [1, 4] and the southern minorities of China [2, 3, 12] were retrieved from published reports on mtDNA variations and were included in our comparative analysis. Principal component analysis (PCA) was conducted using mtDNA haplogroup frequencies and SPSS 16.0 software. The maternal genetic structure of the populations was investigated using the molecular variance approach (AMOVA) [13] with Arlequin 3.11 software (http://cmpg.unibe.ch/software/arlequin3/). The network was constructed by Network software ver.

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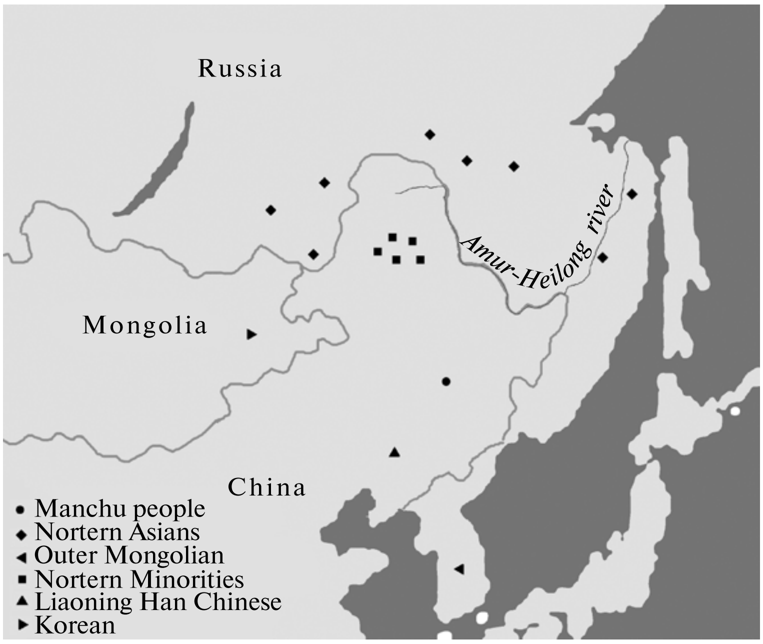

Fig. 1. Geographic distribution of the Manchu people and their neighboring populations.

4.5.1.0 (www.fluxus-engineering.com) using the median-joining method [14].

RESULTS

The mtDNA sequence variations of the 47 individuals are listed in table 1, and fourteen mtDNA haplogroups were found in the samples. In whole, haplogroups A, C, D, G and Z, the northern East Asian-dominating haplogroups [1, 15], reached 68.1% in the Manchus. In contrast, haplogroups B, F and N9a, the southern East Asian-dominating haplogroups [1], averaged to 25.5%. The high frequencies in the Manchu population have haplogroups D4, A and B4, what is very similar to those in the neighboring populations, such as the Inner Mongolians, northern Han Chinese and Duars [1, 6, 16]. Haplogroup C, which has a very high frequency in the Siberian populations [10, 11], was shared by only two individuals in this study.

The result of the PCA of mtDNA haplogroup frequencies is presented in fig. 2. The distribution of the populations in the PCA plotting was in line with their geographic distribution. The northern Asian populations are located in the upper left-hand part of fig. 2; the people who lived in northern China, such as the northern Han people, northern minorities and the Manchu people, are clustered in the upper right-hand part of fig. 2; those who lived in southern China, such as the southern Han people, Tibeto-Burman, Hmong-Mien and the Daic populations, are gathered in the lower right-hand part of fig. 2. The Manchu people are pooled into the northern minorities. They were closest to Duar, Inner Mongolian and Koreans, and were far from the Ewenki and Oroqen.

AMOVA was employed to evaluate the maternal genetic differentiations between the Manchu and other populations (table 2). The F_{st} values of Manchu/northern minorities, Manchu/northern Han and Manchu/Tibeto-Burman (0.00211, 0.00232 and 0.00273) were not significant ($p > 0.05$), indicating that the Manchu were close to those populations in the maternal lineages. In contrast, Manchu and those populations (Daic, Hmong-Mien and Southern Han) living in southern China were significantly different (F_{st} value: 0.04718, 0.04273 and 0.01904 respectively, $p < 0.05$). In addition, F_{st} between the Manchu people and the Northern Asian population was the highest (F_{st} value: 0.06128, $p < 0.05$). To further understand the genetic relationship between the Manchu and their neighboring populations, the genetic differentiations between each of the retrieved populations of northeastern China and the Manchu were investigated by AMOVA. In table 2, the strongest similarity was shown for the Inner Mongolians and the Manchu (F_{st} value, 0.00282, was the lowest, $P > 0.05$) and the F_{st} values of Manchu/Liaoning Han Chinese and Manchu/Korean were almost equal (0.00454 and 0.00516 respectively) and both were not significant, indicating the close maternal relationship among them. On the contrary, the F_{st} values of Manchu/Daur, Manchu/Ewenki and Manchu/Oroqen were significant (0.01350, 0.01486 and 0.02415; $P < 0.05$), what suggests more extensive differentiations between the Manchu and these three populations.

Table 1. Variable sites of HVR-I in 47 Manchu individuals

Samples	Haplogroups	Mutations in HVR-I (16000+)
M1	A	223-290-319-362
M2	A	183C-189-223-290-319-362
M3	A	223-290-319
M4	A	223-287-290-319-362
M5	A	223-263-290-319-362
M6	A	223-290-311-319-362
M7	B4	111-136-183T-189-217-218-239-248
M8	B4	182C-183C-189-217
M9	B4	092-140-182C-183C-189-217-274-311-335
M10	B4	136-183C-189-217-218-239-248
M11	B5	140-187-189-245-256-266G
M12	B5	136-140-183C-189-266A
M13	B5	140-187-189-256-266G
M14	C	223-297-298-311-327
M15	C	223-298-327
M16	D4	223-311-362
M17	D4	223-245-362
M18	D4	092-223-325-362
M19	D4	092-223-325-362
M20	D4	086-223-311-362
M21	D4	223-256-311-362
M22	D4	223-362
M23	D4	223-362
M24	D4	223-274-291-362
M25	D4	223-362-390
M26	D4	174-223-311-362
M27	D4	223-224-245-292-362
M28	D4	092-111-223-362
M29	D4	223-362
M30	D4	223-274-291-362
M31	D4	223-362
M32	D4	129-223-249-327-362
M33	D5	092-164-167-172-182C-183C-189-223-266-272-293-362
M34	D5	164-167-172-182C-183C-189-223-266-362
M35	D5	183C-189-223-362
M36	F1b	111-129-189-304
M37	F1a	108-129-162-172-201-304-311
M38	F1c	289-291-304
M39	F1c	111-129-266-304
M40	G	172-223-327-362
M41	G	093-223-227-234-278-309-362
M42	G	172-223-327-362
M43	M	174-192-223
M44	M7	185-223-260-297
M45	M8	184-189-223-298-319
M46	N9a	093-129-223-245-257A-261
M47	Z	185-223-260-298

Table 2. AMOVA Results for mtDNA

Grouping	F_{st} values	P values
Manchu/Daic	0.04718	0.00000 ± 0.00000
Manchu/Hmong-Mien	0.04273	0.00000 ± 0.00000
Manchu/Northern Han	0.00232	0.20919 ± 0.01372
Manchu/Northern minorities	0.00211	0.23754 ± 0.01304
Manchu/Northern Asian	0.06128	0.00000 ± 0.00000
Manchu/Southern Han	0.01904	0.00196 ± 0.00136
Manchu/Tibeto-Burman	0.00273	0.17204 ± 0.00998
Manchu/Oroqen	0.02415	0.00978 ± 0.00382
Manchu/Ewenki	0.01486	0.04594 ± 0.00696
Manchu/Daur	0.01350	0.04985 ± 0.00696
Manchu/Korean	0.00516	0.18768 ± 0.00988
Manchu/Liaoning Han Chinese	0.00454	0.16716 ± 0.01078
Manchu/Inner Mongolian	0.00282	0.24829 ± 0.01336

To further evaluate the relationship between the Manchus and the neighboring populations, those people living near the Manchu were retrieved to construct a median joining network together with the mtDNA sequences obtained by this study. The results of network analysis are shown in fig. 3. In haplogroup A, M1 shared the central node with one Mongolian, one Daur and four northern Asians, and M3 shared the same haplotype with one Korean. Other individuals M2, M4, M5 and M6 were around the central circle. In haplogroup B4, M8 shared one node with four northern Asians; M7 and M10 were close to two Mongolians; M9 was near one Han Chinese and one Korean, but he was far from the northern Asian populations. In haplogroup B5, three individuals M11, M12 and M13 formed a cluster with Liaoning Han Chinese and Koreans. In haplogroup C, M15 shared the central node with three Mongolians, one Oroqen, one Ewenki, one Daur and many Northern Asians, and M14 was also close to the Northern Asian populations. In haplogroup D4, almost all Manchu individuals presented similar genetic affinity to all the populations retrieved in this study. In haplogroup D5, M33 was close to one Daur; M34 to two northern Asians; M35 to one Liaoning Han Chinesian, one Daur and two Oroqens. In haplogroup F, M36, M37 and M39 were close to Li-

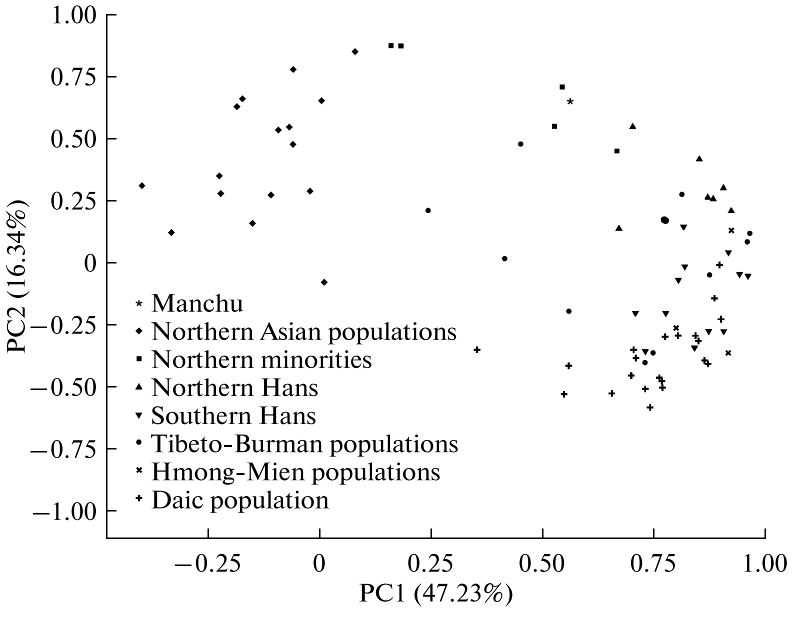

Fig. 2. Principal component analysis of the mtDNA haplogroup frequencies of the Manchu people and related populations.

aoning Han Chinese and Mongolians, and M38 to the northern Asians. In haplogroup G, M40 and M41 were close to five Oroqens, and M42 to one Northern Asian and one Korean. In haplogroup M7, M44 was closest to the Mongolians, Liaoning Han Chinese, Koreans, Daurs and northern Asians. In haplogroup M8, only Liaoning Han Chinese, Koreans, Mongolians and M45 were shown, and M45 shared the same node with two Koreans, indicating a close genetic relationship among them. In haplogroup N9a, M46 was close to Mongolians, Daurs and northern Asians. In haplogroup Z, M47 shared a central haplotype with one Liaoning Han Chinese, two Ewenkis, three Koreans and one northern Asian.

DISCUSSION

Most of the Manchu people lived in northeastern China near Siberia. But a close relationship between the Manchus and Northern Asian populations, however, was not found by genetic analysis. a) The 16 haplogroups of Manchu people showed the representative character of East Eurasians, whereas most of the northern Asians contained the specific European haplogroups more or less [10, 11]. Among the modern populations of Siberia, there is a very high frequency of haplogroup C [10, 11]. In contrast, the frequency of haplogroup C in Chinese is low [1–4, 12]. In Manchu, only two individuals shared haplogroup C. b) In the PCA plot, the Manchu are far away with all the Northern Asian populations and the northern minorities such as Ewenki and Oroqen, originating from North Asia. c) AMOVA analysis showed that the divergence between the Manchu and Northern Asian populations was significant ($F_{st} = 0.06128$; $P = 0$). d) Most of the Manchu people presented a close relationship with the Mongolians and the Han Chinese in network analysis. There is still a fact that some Manchu individuals shared the same haplotypes with northern Asians, what implies that the Manchu originated from the Amur-Heilongjiang River valley.

In contrast, PAC and AMOVA analysis showed that the Manchu were close to the neighboring populations in the maternal lineages: a) Manchu pooled into the northern minorities, and was also close to some northern Han populations in the PCA plot; b) the F_{st} values of Manchu/Northern minorities and Manchu/Northern Han (0.00211, 0.00232) both were low, and the differentiations among them were not significant ($P > 0.05$). It should be pointed out that the Manchu were also near the Tibeto-Burman populations in the AMOVA analysis (F_{st} value: 0.00273; $P > 0.05$). According to historical records, the Tibeto-Burman populations derived from the ancient Di-Qiang tribes, the ancestors of the northern Han Chinese [2, 7]. This indicates a close relationship between the Tibeto-Burman populations and the northern Han Chinese, what further supports that the Manchu were close to the northern Han Chinese.

According to historical documents the Manchu originally lived in the Amur-Heilong River valley. During the rise of the Manchu, they gradually migrated southward to the southern area of northeastern China, and finally, they came to power in China and founded the Qing Dynasty in the seventeenth century. When they ruled China, the Manchu ethnicity largely mixed with the Han Chinese [7]. During this time the Manchu also mixed with other neighboring populations. For example, many Manchu males married female Inner Mongolians, which was just proved by the result of AMOVA analysis. The F_{st} value of Manchu/Inner Mongolian was the lowest (0.00282), suggesting that Manchu bore a high similarity to the Inner Mongolians. Moreover according to the AMOVA analysis the Manchu were also close to the northern Han Chinese and Koreans in maternal lineages (F_{st} value of Manchu/Han in northeastern China: 0.00454; F_{st} value of Manchu/Koreans:0.00516). In network analysis, almost all of the Manchu samples were always near the Mongolian and Liaoning Han Chinese, showing that they shared a genetic similarity in the female lineages. On the contrary, the Manchu shown to be highly different from the Ewenki, Oroqen and Daur, who lived in the neighboring area from where the Manchu originated (The F_{st} values of Manchu/Daur Manchu/Oroqen and Manchu/Ewenki were 0.01350, 0.01486 and 0.02415, and all of P values <0.05). This is in line with the fact that the Manchu migrated southward and gradually mixed with the natives.

In conclusion, the Manchu are far from the people, who lived in the cradle of the Manchu, and are close to those, who live together with the Manchu now, suggesting that the Manchu gradually mixed with the natives following their southward migration.

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