



Molecular genealogy of Tusi Lu's family reveals their paternal relationship with Jochi, Genghis Khan's eldest son

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Abstract

Genghis Khan's lineage has attracted both academic and general interest because of its mystery and large influence. However, the truth behind the mystery is complicated and continues to confound the scientific study. In this study, we surveyed the molecular genealogy of Northwestern China's Lu clan who claim to be the descendants of the sixth son of Genghis Khan, Toghan. We also investigated living members of the Huo and Tuo clans, who, according to oral tradition, were close male relatives of Lu clan. Using network analysis, we found that the Y-chromosomal haplotypes of Lu clan mainly belong to haplogroup C2b1a1b1-F1756, widely prevalent in Altaic-speaking populations, and are closely related to the Tore clan from Kazakhstan, who claim to be the descendants of the first son of Genghis Khan, Jochi. The most recent common ancestor of the special haplotype cluster that includes the Lu clan and Tore clan lived about 1000 years ago (YA), while the Huo and Tuo clans do not share any Y lineages with the Lu clan. In addition to the reported lineages, such as C3*-Star Cluster, R1b-M343, and Q, our results indicate that haplogroup C2b1a1b1-F1756 might be another candidate of the true Y lineage of Genghis Khan.

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Introduction

Genetic genealogy is the application of genetics to traditional genealogy to detect the genetic relatedness between individuals through the use of DNA testing, with a specific emphasis on Y-chromosomal markers. Because many societies are patriarchal and define family relations through the male line, the Y chromosome in conjunction with oral and documentary records is a superb tool to research a family's genetic lineage [1]. The study of historical persons' Y lineages is a particularly active area in genetic genealogy. Geneticists have studied the lineage of Thomas Jefferson of America [2], Napoléon and the House of Bourbon of France [3, 4], the Romanov family of Russia [5], Genghis Khan, the House of Aisin Gioro, and Emperor CáoCào of China [6–8].

Among them, the Y lineages of Genghis Khan and Giocangga were first inferred by special Y-chromosomal haplotypes, the so-called 'star cluster' (equal to haplogroup C3b1a3a1-F3796) and the 'Manchu cluster' (equal to haplogroup C3b2a-M48), which occur with a high frequency in Mongolic and Tungusic speaking populations [7, 9]. However, without genealogical support, the veracity of

these two cases are debatable. In recent studies, we found that the males of the House of Aisin Gioro possessed a rare haplotype that belongs to the haplogroup C3b1a3a2-F8951 [10], irrelevant to the C3b2a-M48 ‘Manchu cluster’ [9]. The association with Genghis Khan and the ‘star cluster’ is also unclear [11, 12].

The search of the true Y lineage of Genghis Khan has been fraught with difficulties. Archeologists have only discovered a few Mongol noble cemeteries, let alone a tomb belonging to the Kha’s family, in large part due to the custom of keeping ancestral graves a secret [13]. Additionally, the few ancient DNA studies have produced contradicting results. Judging from the genetic data retrieved from the skeletal remains of the assumed Golden family graves in Tavan Tolgoi (Eastern Mongolia) [14], Lkhagvasuren has suggested that Genghis Khan and his family belong to haplogroup R1b-M343, while another investigation from a high-status noble tomb in Hebei (Northern China) indicates that haplogroup Q is another possibility [15]. Aside from the archeological evidence, the analysis of the DNA of selected living relatives has produced large discrepancies between the Y lineages of different family lines [16]. For instance, three remaining family lines of Genghis Khan belonged to the different subhaplogroup C3; in addition, the Y lineages of Khasar (Genghis Khan’s full brother) and Belgutei (Genghis Khan’s paternal half-brother) have been identified as haplogroup O3 and haplogroup R1a1a, respectively. This may be the result of unrecorded adoption, surname changes, and nonpaternity events.

Imperial officials authorized the Tusi, tribal leaders mainly located in Northwestern and Southwestern China during the age of Yuan, Ming, and Qing dynasties, to provide troops, suppress local rebellions, and periodically pay tribute to the rulers in Beijing. The Lu family was one of the illustrious Tusi families that exercised great influence over various aspects of the economy and society of the Gansu–Qinghai area in Northwestern China. According to the historical records and genealogy books compiled in Qing dynasty, such as *Draft History of Qing - Biography of the Tusi in Gansu Province* (清史稿-甘肃土司传), *Renewal of the Lu Clan’s Genealogy* (重续鲁氏家谱), and *Family Genealogy of the Lu Clan* (鲁氏世谱), their common ancestor can be traced back to Toghan (脱欢; ?-1371 AD), the descendant of Khulgen (阔列坚; the sixth son of Genghis Khan). During the late Yuan and early Ming dynasty, Toghan and his tribe became subjects of the Ming government and were awarded the Han-Chinese surname Lu for their service and gallantry in combat. Since then, the Tusi Lu family were garrisoned the Liancheng town, Yongdeng county, Northwestern China, for 19 generations, around 560 years. Moreover, on the basis of their oral tradition, the Huo and Tuo clans are the descendants of

HuoShi (火石; Toghan’s son) and TuoChi (脱赤; Toghan’s younger brother). Thus, the ancestry of these three clans, the Lu, Huo, and Tuo, can provide insight into the genetic lineage of Genghis Khan.

Materials and methods

We collected oral samples from male individuals of the Lu clan ($n = 30$), Huo clan ($n = 31$), and Tuo clan ($n = 30$). Among the Lu clan, 22 individuals have complete genealogical records inside the stemma of Tusi Lu, while the remaining donors may not be the descendants of Tusi Lu but rather of their retinue. This study has been approved by the Ethics Committee for Biological Research at Fudan University and all the samples were collected with informed consent. For each samples, we extracted DNA, typed relevant Y-chromosomal SNPs via a hierarchical strategy, as we did in previous studies [17, 18], and amplified 17 Y-STRs (DYS19, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439, DYS448, DYS456, DYS458, DYS635, Y-GATA H4, and DYS385a/b) using the Y-filer kit (Life Technologies, Carlsbad, CA, USA). The network of the samples belonging to the targeted lineage and comparative samples was drawn using Network 4.6.1.2 (Fluxus) with a median joining algorithm. Time estimation for each group was made using both ASD and BATWING methods based on 15 STRs (excluding DYS385a/b) [17]. A generally accepted generation time of 25 years was used to produce a time estimate in years.

Results and discussion

A total of 91 male individual samples’ Y-chromosomal haplotypes (Table S1) were assigned to 11 distinct haplogroups (Fig. 1) according to the nomenclature of Y-chromosome consortium and ISOGG Y-DNA haplogroup Tree 2017, Version:12.334. Haplogroups C2b1a1b1-F1756 and C2c1*-F2613+, M407-, which are prevalent in Altaic-speaking populations and northern Han populations, respectively [19]. Together, this accounts for 83% of the Y-chromosome variation in the samples from the Lu clan. Aside from C3*-Star Cluster (the proposed lineage of Genghis Khan), C2b1a1b1-F1756 is another important paternal lineage under haplogroup C3*-M217, which is observed at high frequencies in populations across the eastern region of the Mongolian Plateau with moderate frequencies in Altai (6.7%), Teleut (9.1%), Uzbek (7.1%), and Kalmyk populations (6.1%) [19]. Notably, the majority of the individuals (75%) who claimed to be the descendants of Tusi Lu’s attendants were assigned as the haplogroup C2c1*-F2613+, M407-, while 16 of the

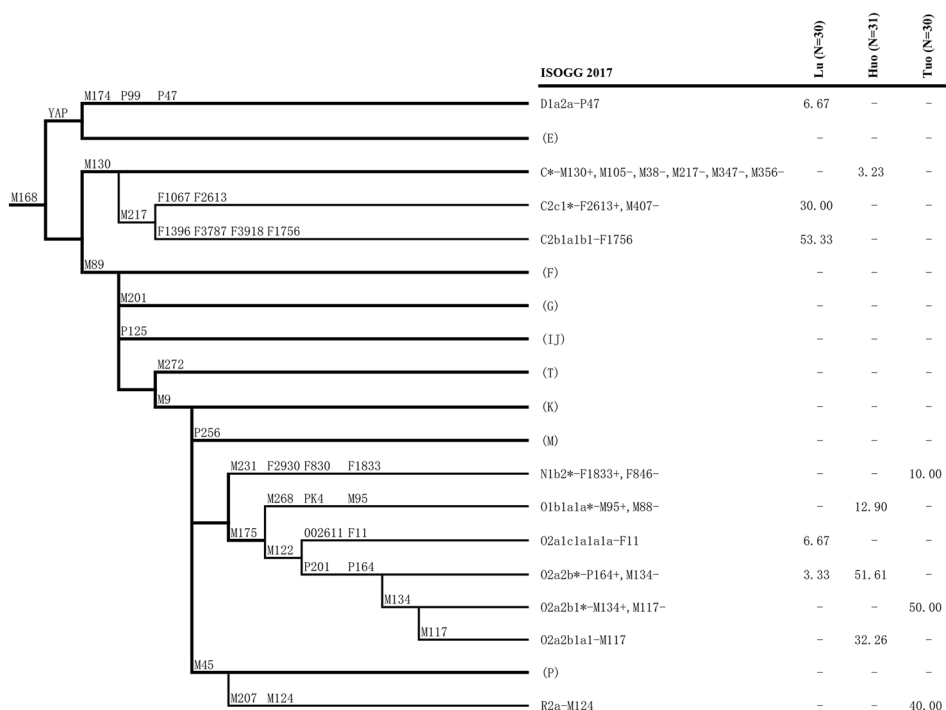


Fig. 1 The phylogenetic relationship of Y-chromosome haplogroups surveyed in this study and their frequencies in Lu clan, Huo clan, and Tuo clan. The marker names are shown along the branches, and haplogroup names are shown on the right side according to the ISOGG

Y-DNA Haplogroup Tree 2017. Potentially paraphyletic undefined subgroups are distinguished from recognized haplogroups by the asterisk symbol. Haplogroups tested for but not seen in this study are enclosed in parentheses

22 samples recorded in the genealogy book were C2b1a1b1-F1756.

Thus, it is quite possible that the paternal lineage of Tusi Lu's family belong to the haplogroup C2b1a1b1-F1756. Haplogroup O2a2b*-P164+, M134-, which is found at moderate or relatively low levels in Han Chinese [20], exhibits a high frequency (51.61%) in the samples from the Huo clan. The frequencies of eastern Asia and southern China-specific haplogroups (O2a2b1a1-M117 and O1b1a1a*-M95+, M88-) [21] in Huo clan are 32.26% and 12.9%, respectively. The common haplogroup in Han Chinese, O2a2b1*-M134+, M117-, in south Asian/western Eurasian, R2a-M124, and in Sino-Tibetan speaking populations, N1b2*-F1833+, F846-, has also been found in the Tuo clan samples at a frequency of 50%, 40%, and 10%, respectively [21, 22].

Accordingly, there is no patrilineal relationship between the Lu clan and the other two clans. The mistake in the oral tradition may come from the association of their ancestors' Chinese names. Toghan (脱欢) and TuoChi (脱赤) both of which have the Chinese family name “脱”. HuoShi (火石) and TuoChi (脱赤), which share the same meaning “red or fire” of the given name. Nevertheless, it seems more likely that they were Anda, a Mongolian term for friends, and allies that literally means “as though born from the same womb”.

To clarify the origin of Tusi Lu's family, we compared the Y-STR haplotypes that belong to the haplogroup C2b1a1b1-F1756 against the largest Y-STR dataset, Y-chromosome haplotype reference database (YHRD), and conducted a median-joining network analysis on the relevant haplotypes collected from the literature (Table S2) [19]. Although searching the YHRD did not produce any positive results, surprisingly, the Tore clan haplotypes drawn from the Kazakhstan DNA project are close to the Y profiles of the Tusi Lu's family [23]. Tore is a noble clan in the Kazakhs. The common ancestor of Kazakh Tore is Khan Zhanybek, who is the tenth generation down from Jochi, the eldest son of Genghis Khan [23]. As shown in Fig. 2, all 16 samples from the Lu clan, four samples from the Tore clan, and some samples from Han ($n = 2$), Tibetan ($n = 1$), Buryats ($n = 2$), Kyrgyz ($n = 1$), Manchu ($n = 1$), Hui ($n = 2$), and Hazaras ($n = 1$) form a defined cluster (Cluster I, in which samples can be linked to each other within one-step difference) in the upper-left part of the network (also see Table S2).

This pattern of Cluster I is understandable. Genghis Khan (c.1162–1227) conquered the most of Eurasia and established the largest land empire in history. Although the Mongol empire soon disintegrated as a cohesive political unit, he and his close male relatives' descendants ruled this large area for many generations. Our network analyses show that these male-line descendants blended into several

Fig. 2 Median-joining networks of Y-STR haplotypes based on 13 Y-STRs (Table S2) for the haplogroup C2b1a1b1-F1756. Haplotypes are represented by circles with area proportional to the number of individuals. Colors indicate the geographic origin

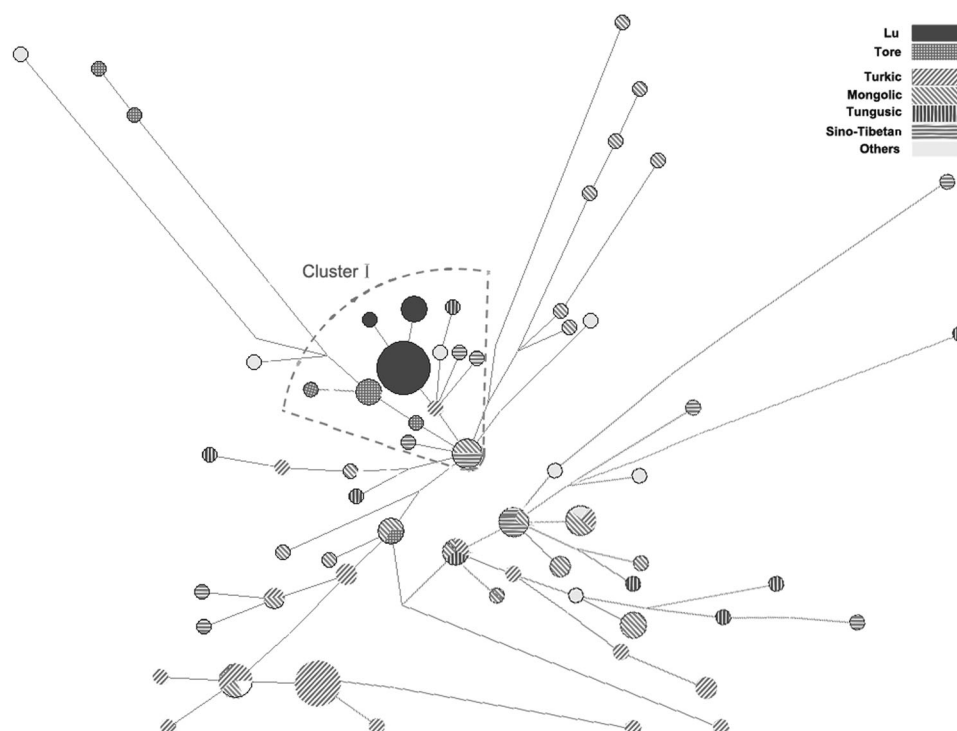


Table 1 The coalescence time estimations for each group using both BATWING and ASD method (time in years)

	Group	OMRB		OMRS		lmMR		EMR	
		TMRCA	95 % CI	TMRCA	95 % CI	TMRCA	95 % CI	TMRCA	95 % CI
BATWING	Lu clan	448.3	37.6–3888.9	361.3	29.7–3199.1	469.6	39.6–4064.1	1792.5	152.7–16159.1
	Cluster I	1807.6	384.0–8906.6	1447.5	315.2–7052.6	1835.6	389.5–9019.5	7154.1	1070.5–47877.1
	hg C2b1a1b1-F1756	6128.8	910.5–40286.8	5587.6	796.7–38461.2	5785.9	840.5–39978.0	20453.2	2881.6–147362.5
ASD		TMRCA	SE	TMRCA	SE	TMRCA	SE	TMRCA	SE
	Lu clan	148.1	235.0	79.8	123.1	189.8	296.5	696.8	967.9
	Cluster I	975.0	740.2	955.7	809.7	1027.5	784.1	4225.6	2965.8
	hg C2b1a1b1-F1756	2128.6	664.0	1934.2	557.1	2371.2	1083.2	9714.1	5263.1

ASD average squared distance; BATWING Bayesian analysis of trees with internal node generation; OMRB and OMRS Two observed genealogical mutation rates; lmMR Genealogical mutation rate adjusted for population variation using logistic model; EMR the evolutionary mutation rate

populations, roughly in accord with historical events. For instance, after the fall of the Yuan dynasty, those who surrendered themselves to the Ming, such as Tusi Lu's family, became Han Chinese. Likewise, in 1207, Jochi conquered the Buryats in Siberia, and in 1210 and 1218, he waged wars on the Kyrgyz, a potential reason why the Cluster I samples could be found in these populations. Moreover, because the aristocratic marriage between Manchu and Mongolian was a basic policy of Qing dynasty, it is natural that we found the Cluster I sample in Manchu. The Khoshut tribes of Oirat people, whose founder was Habutu Hasar, Genghis Khan's full brother, settled around Qinghai

Lake during the 17th and 18th centuries. After Khoshut Khanate's fall, some of the Khoshuds adopted Tibetan culture while others became Hui Muslims. Notably, we also found a Cluster I sample in the Hazaras. Many of the Hazaras claimed descent from Genghis Khan according to their oral history. Therefore, scholars have considered this as evidence that the true Y lineage of Genghis Khan was C3*-Star Cluster [6].

Subsequently, we estimated the coalescence time for the Lu clan, Cluster I, and all haplogroup C2b1a1b1-F1756 individuals (Table 1). In our previous case studies, we evaluated the Y-STR dating in deep-rooting pedigrees and

we found that Y-chromosomal genealogical mutation rates (OMRB and ImMR) in BATWING method can give the best-fit estimation for dating a historical lineage [24]. In this research, OMRB and ImMR rate traced the Lu clan's common ancestor to 448.3 and 469.6 years ago (YA), respectively, in the Ming dynasty. These dates are somewhat younger than the records of genealogy (the real living time of Toghan is about 600 YA). OMRS rate and ASD estimations have underestimated the time at several hundred years shorter. For Cluster I's TMRCA estimate, ASD gave 1027.5 YA (ImMR), 975 YA (OMRB), and 955.7 YA (OMRS) almost identical to the real living time of Genghis Khan and his close male relatives, whereas BATWING gives 1835.6 (ImMR), 1807.6 (OMRB), and 1447.5 YA (OMRS), which are older than the published estimate of C3*-Star Cluster (~1000 YA [6] or 925 YA [25]). All haplogroup C2b1a1b1-F1756 individuals' BATWING estimations gave an age range of 5587–6126 YA; this is coherent with the C2b1a1b1-F1756 expansion since about 5500 YA calculated by using the whole Y-chromosome sequences [19]. Seemingly, ASD give the best-fit estimation for the Lu clan's TMRCA estimation; BATWING gives the best-fit estimation for Cluster I's TMRCA estimation but with larger confidence intervals. In addition, in the Kazakhstan DNA project, four haplogroup R2 samples of Kazakh Tore have more than ten steps of Y-STR mutations than the Tuo clan, which excludes the possibility that they shared a common ancestor with the past 1000 years.

According to *The Secret History of the Mongols*, the first wife of Genghis Khan was once captured by a Merkit tribe for a few months. She was then recovered by Genghis Khan (known as Temüjin at that time) and gave birth to Jochi shortly afterward. Therefore, the identity of Jochi has always been a longstanding issue. Nevertheless, the molecular genealogy of Tusi Lu's family linked their paternal relationship with the Tore clan, descendants of Jochi. This suggests that, in addition to the reported lineages C3*-Star Cluster, R1b-M343, and haplogroup Q [6, 14, 15], the haplogroup C2b1a1b1-F1756 is another viable candidate for the true Y lineage of Genghis Khan.

Although ancient DNA results are necessary for definitively clarifying the true Y lineage of Genghis Khan, previous studies have made some progress in exploring this genetic history by using molecular tools. In this study, we found that Tusi Lu's family, the descendant of Khulgen, and the sixth son of Genghis Khan belonged to the Altaic-speaking population specific haplogroup C2b1a1b1-F1756. However, thousands of miles away in Kazakhstan, the Y lineage of Tore clan who traced their common ancestor to Jochi, the first son of Genghis Khan, is also from the haplogroup C2b1a1b1-F1756. Importantly, their ASD estimations using a genealogical rate are ~1000 YA, which is

closer to when Genghis Khan and his male relatives were alive.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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