

Y-chromosome evidence for no independent origin of modern human in China

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Abstract East Asia is one of the few regions in the world where a large number of human fossils have been unearthed. The continuity of hominid fossils in East Asia, particularly in China has been presented as strong evidence supporting an independent origin of modern humans in this area. To search for such evidence of a possible independent origin of modern humans in China, a total of 9988 male individuals were sampled across China. Three Y-chromosome biallelic markers (M89, M130 and YAP), which were located at the non-recombinant region of Y-chromosome, were typed among the samples. Our result showed that all the individuals carry a mutation at one of the three loci. The three mutations (M89T, M130T, YAP⁺) coalesce to another mutation (M168T), which was originally derived from Africa about 31000 to 79000 years ago. In other words, all Y-chromosome samples from China, with no exception, were originally derived from a lineage of African origin. Hence, we conclude that even a very minor contribution of *in situ* hominid origin in China cannot be supported by the Y-chromosome evidence.

Keywords: Y-chromosome, haplotype, SNPs, modern human origin, Chinese.

Modern human origin and migrations in China is becoming a hot topic recently due to new evidence from genetic studies^[1,2]. In fact, the debate on this subject has lasted in the past decade between anthropologists and geneticists. Broadly speaking, there are two competing hypotheses on the origin of modern humans in China: the “Out-of-Africa” hypothesis and the multiregional hypothesis. Both agree that *Homo erectus* originated in Africa and expanded to other continents including China about one million years ago. But they differ in the origin of modern human (*Homo sapiens sapiens*). The first hypothesis supported by geneticists proposes that a second migration out of Africa happened about 100000 years ago,

when the anatomically modern humans of African origin conquered the world including China by completely replacing the archaic human population (*Homo sapiens*)^[3,4]. However, the multiregional hypothesis supported by some archaeologists stated that independent origins or shared multiregional evolution with continuous gene flows between continental populations occurred in the past millions of years since *Homo erectus* came out of Africa^[5,6]. A compromised version of the “Out-of-Africa” hypothesis emphasizes the African origin of most human populations but leaves space for possible minor local contributions^[7–10].

East Asia is one of the few regions with relatively abundant hominid fossils that span the last several hundreds of thousands years, and was claimed to have the continuity in morphology from *Homo erectus* to *Homo sapiens* and *Homo sapiens sapiens*. Such evidence suggests the possibility of independent *in situ* evolution. However, almost all of the genetic evidence supports the “Out-of-Africa” hypothesis which was first proposed in the late 1980s. In a recent study of Asian population, 30 autosome microsatellite markers were typed in the extent of Chinese population. It was suggested that modern humans in China originated in Africa and probably entered from the southern part of East Asia^[1]. Although Chu’s study cannot exclude the possibility of *in situ* origin, it supports the “Out-of-African” hypothesis of modern human origin in China. However, due to the high mutation rate of the microsatellites, Chu’s study fell short in providing unequivocal evidence on modern human origin in China. Recently Su et al.^[2,11] reported an extensive survey in East Asian populations using Y-chromosome biallelic markers. The biallelic markers on the Y-chromosome usually occurred only once during evolution and therefore are more stable than the microsatellite loci. The markers on the non-recombinant part of the Y-chromosome allow the reconstruction of intact haplotypes, which are not likely to be eroded by recombination and recurrent mutation and, therefore, highly informative for tracing ancient human migration. Given the fact that the Y-chromosome has a smaller effective population size than that of the autosomes, it is more sensitive to population bottleneck events, which usually were associated with migrations. Su et al.^[2,11] employed a set of 19 Y-SNP biallelic markers to investigate their haplotype distribution in Chinese population, and confirmed that modern human in East Asia originated from Africa, and entered East Asia from the south, and a northward migration led to the peopling of northern China, and the entrance was estimated to be 18000 — 60000 years ago.

In order to test the hypothesis of modern human origin, we typed three root Y-chromosome polymorphisms (M89, M130 and YAP) in 9988 male samples across

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China. These three polymorphisms (M89T, M130T, YAP⁺) coalesced to another mutation (M168T), which was introduced into human population about 31000—79000 years ago. Furthermore, it was shown that M168T was originally derived from Africa, and no single individual was found that carried a lineage older than M168T outside Africa^[12]. Hence, the three markers can be used to test the completeness of replacement of modern humans of Africa origin in East Asia. As predicted by the “Out-of-Africa” hypothesis, an observation of a male individual not having one of the three polymorphisms would be an indication of a potential local origin and possibly lead to the rejection of such completeness.

1 Materials and methods

(i) Sample collection and DNA extraction. A total of 9988 male samples were collected covering 29 provincial areas in China, including 9 ethnic nationalities. Three Y-chromosome biallelic markers (M89, M130 and YAP) were genotyped in all the 9988 samples. The geographical origins of 2924 individuals were assigned based on the birthplaces of their grandparents. Genome DNA was extracted using the standard phenol-chloroform method. DNA samples were stored at -20°C after extraction.

(ii) Genotyping of Y-SNPs. Genotyping of YAP was conducted by agarose gel electrophoresis directly after PCR, and M130, M89 were typed using a PCR-RFLP assay. The restriction sites were engineered for M130 (*Bsl* I) and M89 (*Nal* III) by designing mismatched primers. Primers sequences of three Y-SNPs are listed in table 1. Each Y-SNP was amplified using 10 μL PCR system, including 20 ng/μL DNA template, 2 pmol of each primer, 2.5 mmol/L 4 × dNTP, 1 μL 10 × PCR buffer, and 0.5 unit of Taq DNA polymerase with PCR reaction condition: denaturing at 94°C for 3 min; 94°C for 25 s → 56°C for 25 s → 72°C for 25 s, 35 cycles; elongating at 72°C for 5 min. Restriction enzymes were purchased from New England BioLabs.

2 Result

In a total of 9988 samples typed, with no exception, all of them have one of the derived alleles at the three loci, YAP⁺, M89T and M130T. Among them, the frequency of the individuals carrying M89T, M130C, YAP⁻ is 93.4%, the predominant haplotype in the samples studied. The other two haplotypes, M89C, M130T, YAP⁻ and M89C, M130C, YAP⁺, account for 3.7%, and 2.9% respectively. Among the 9988 samples, there are 2924 samples with reliable geographic and nationality information, which

Table 1 Information of the 3 Y-loci typed and the primer sequences

	M89	M130	YAP
Site polymorphism	C→T mutation	C→T mutation	Alu insertion
forward	Tcctatgaggtgcatgaattg	Tatctcctctctattgcag	Actgctaaaaggggatggat
reverse	Gcaagtcaggcaaatgtagacat	ccacaaggggaaanaaacac	Gaggggaagataaagaata
Length of PCR product	116 bp	205 bp	150 bp, 350 bp
RE enzyme	<i>Nla</i> III	<i>Bsl</i> I	\
Enzyme	5'.....▼C[ATG].....3'	5'.....[CN]N▼	\
Digestion site		NNNNGG.....3' N	\
Polymorphic length	M89C: cut (22 bp + 94 bp) M89T: no cutting	M130C:cut (162 bp+43 bp) M130T: no cutting	YAP ⁺ 350 bp YAP ⁻ 150 bp

Table 2 Frequency distribution of three Y-SNPs in China

Region	Sample size	M89T	Frequency (%)	M130T	Frequency (%)	YAP ⁺	Frequency (%)
Northeast	477	442	92.7	22	4.6	13	2.7
North	1013	945	93.3	49	4.8	19	1.9
Northwest	433	398	91.9	10	2.3	25	5.8
Middle	829	792	95.5	24	2.9	13	1.6
South	172	154	89.5	3	1.7	15	8.7
Total	2924	2730	93.4	108	3.7	85	2.9

Table 3 Frequency distribution of three Y-SNPs in some ethnic nationalities in China

Nationality	Sample size	M89T	Frequency (%)	M130T	Frequency (%)	YAP ⁺	Frequency (%)
Bai	32	27	84.4	3	9.4	2	6.25
Tibet	30	25	83.3	1	3.3	4	13.3
Dai	52	46	88.5	0	0	6	11.5
Hui	69	63	91.3	2	2.9	4	5.8
Han	2686	2517	93.7	101	3.8	68	2.5

allow us to analyze the distribution of the three Y markers in different ethnic populations and its implication for population history in China (see tables 2 and 3 for frequency distribution). In general, there are no significant differences of haplotype distribution among different geographic areas. The YAP⁺ is more prevalent in southern and northwestern China than other areas. According to the previous report^[13–16], Tibetans have the highest frequency of YAP⁺ in East Asia. Therefore, the higher frequency of YAP⁺ in southern and northwestern populations indicates the influence of Tibetan populations in recent times. The M130T, an Asian specific allele, has a wide distribution in East Asia, reaching the highest frequency in northern Asia (62.6% in Siberians). A cline of M130T was observed from north to south in East Asia.

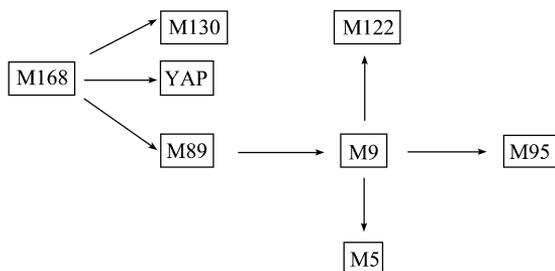


Fig. 1. Systematic evolution of some Y-SNPs.

3 Discussion

As we mentioned above, a continuous evolutionary chain has been proposed to connect *Homo erectus* to *Homo sapiens sapiens* in China, and this was used to support an independent origin of modern humans in East Asia. However, the interpretation of the morphological continuity is still controversial. Furthermore, a close examination of the collection of hominid fossils in China revealed a non-trivial temporal gap between archaic humans (*Homo sapiens*) and modern humans (*Homo sapiens sapiens*)^[2]. All the *H.sapiens* fossils are at least 100000 years old, whereas all the *H.s.sapiens* fossils are less than 40000 years old (mostly between 10000–30000 years old). In other words, no hominid fossils that can be dated from 100000 to 40000 years ago have been found in East Asia. The long duration of the temporal discontinuity of the fossil records in China and the distinctive morphological character of the hominid fossils found before 100000 years ago and after 40000 years ago implies that this gap cannot be casually attributed to a missing link. Instead, the extinction of local archaic humans and the subsequent peopling of modern humans from Africa is a more reasonable explanation. Interestingly, this 60000-year fossil gap coincides with the last ice age, during which modern humans of African origin are thought to have arrived in the southern part of East Asia. With receding of glacier, a northward migration led to the first entry of modern humans into southern China and a total replacement of archaic human populations. The com-

pleteness of this replacement was supported by a survey of near 10000 individuals in this study ($P = 5.4 \times 10^{-6}$ assuming a frequency of 1/1000 local contribution)^[1,17,18].

In conclusion, the evidence from Y-chromosome biallelic markers is consistent with the “Out-of-Africa” hypothesis, as all of the haplotypes found in modern Chinese are derived from a small number of African ancestors and no ancient local contributions were found. The replacement of modern humans of African origins in East Asia seems to be complete, leaving the multiregional hypothesis a remote possibility.

Acknowledgements We thank those who provided DNA samples including Wu Dongqi from TSBIO Science & Technology Co. Ltd; Liu Jie from the Fourth Military Medical University, Zheng Shu and Cai Shanrong from Zhejiang University, Zhu Dingliang from Ruijing Hospital and Shanghai Second Medical University, Song Xiufeng, Li Hui and Xu Xiaoyun from Fudan University. This work was supported by the National Natural Science Foundation of China (Grant No. 39993420).

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(Received December 27, 2000; accepted February 26, 2001)