

SHORT REPORT

# An updated tree of Y-chromosome Haplogroup O and revised phylogenetic positions of mutations P164 and PK4

Shi Yan<sup>\*,1,2</sup>, Chuan-Chao Wang<sup>1</sup>, Hui Li<sup>1</sup>, Shi-Lin Li<sup>1</sup>, Li Jin<sup>\*,1,2</sup> and The Genographic Consortium<sup>3</sup>

Y-chromosome Haplogroup O is the dominant lineage of East Asians, comprising more than a quarter of all males on the world; however, its internal phylogeny remains insufficiently investigated. In this study, we determined the phylogenetic position of recently defined markers (L127, KL1, KL2, P164, and PK4) in the background of Haplogroup O. In the revised tree, subgroup O3a-M324 is divided into two main subclades, O3a1-L127 and O3a2-P201, covering about 20 and 35% of Han Chinese people, respectively. The marker P164 is corrected from a downstream site of M7 to upstream of M134 and parallel to M7 and M159. The marker PK4 is also relocated from downstream of M88 to upstream of M95, separating the former O2\* into two parts. This revision evidently improved the resolving power of Y-chromosome phylogeny in East Asia.

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## INTRODUCTION

The phylogeny of Y-chromosome provides a powerful tool to reconstruct history of human populations and paternal pedigrees.<sup>1–3</sup> The dominant group of Y chromosome in Eastern Eurasia is Haplogroup O-M175, comprising roughly 75% of the Chinese<sup>4–6</sup> and more than half of the Japanese population.<sup>7–9</sup> Albeit the huge population of Haplogroup O, its phylogeny<sup>10</sup> is much less adequately resolved than those of Haplogroup R and E, despite the improvements of O tree made during the recent years.<sup>10–12</sup> The most important part of potential interest was the paragroup O3a\*-M324(xM121, M159, M164, M7, M134), which comprises a substantial part of Chinese population (typically 15–50% of Han Chinese) but could not be further resolved in the last few years. Fortunately, recent data from HapMap project (<http://hapmap.ncbi.nlm.nih.gov/>)<sup>13</sup> and certain companies show that several single-nucleotide polymorphisms (SNPs) can further distinguish the paragroup O3a\*. For example, a novel clade defined by IMS-JST002611 (in short, 002611) may count for up to 21% of the East Asians.<sup>4,8</sup>

From the HapMap project, several SNPs were recognized under Haplogroup O-P191 (rs16980601). One of them, rs17269396, was reported as L127.1 on FamilyTreeDNA (<https://www.familytreedna.com/advanced-snp-descriptions.aspx>) as within O3a-P93. Two further SNPs were reported on DecodeMe (<http://demo.decode.me/ancestry/male-line-advanced/O>), which showed that the derived allele has a substantial proportion in all tested samples belonging to Haplogroup O: rs17276338 (25%) and rs17316007 (P164, 30%), indicating their importance. However, their phylogenetic positions relative to other SNPs were not reported.

The SNP P164 does not agree to the position in the latest Y-chromosome phylogenetic tree as defined in the previous study<sup>10</sup> – derivative of SNP M7. Several publicly available individual samples (eg, from 23andMe, <https://www.23andme.com/>) have derived alleles of both M134 and P164, whereas M134 and M7 are parallel subclades. Another SNP, PK4, defining the clade O2a1a, was first discovered in Pakistan.<sup>14</sup> We found that several samples were typed as derivative for PK4 but ancestral for M95 (defining O2a), showing inconsistency with the current tree. Therefore, the position of P164 and PK4 needed to be revised.

## MATERIALS AND METHODS

To revise the phylogeny of Haplogroup O, we collected whole blood samples from 361 unrelated Han Chinese male volunteers at Fudan University in Shanghai, with informed consent. The origins of volunteers can be traced back from all over China, although the majority are from East China, that is, Jiangsu, Zhejiang, Shanghai, and Anhui. DNA was extracted from the samples, and then genotyped at M175, M119, P203, M110, M268, P31, M95, M88, PK4, M176, M122, M324, M121, M164, P201, M159, M7, M134, M117, 002611, P164, L127 (rs17269396), KL1 (rs17276338), and KL2 (rs17323322; KL1 and KL2 are named in this study), using the SNaPshot multiplex kit (ABI, Carlsbad, CA, USA) as described before.<sup>15</sup> Besides the previously listed primers,<sup>10</sup> the primers of newly typed SNPs were designed (Table 1).

## RESULTS

In the updated tree of Haplogroup O (Figure 1), the new lineage names were proposed according to the nomenclature rules.<sup>16</sup> The SNPs L127, KL1, and KL2 are found upstream to M121, M164, and 002611, and together define Haplogroup O3a1. The position of P164

<sup>1</sup>State Key Laboratory of Genetic Engineering and Ministry of Education, Key Laboratory of Contemporary Anthropology, School of Life Sciences and Institutes of Biomedical Sciences, Fudan University, Shanghai, China; <sup>2</sup>Group of Computational Genetics, CAS-MPG Partner Institute for Computational Biology, SIBS, CAS, Shanghai, China  
\*Correspondence: Professor Dr L Jin or Dr S Yan, State Key Laboratory of Genetic Engineering and Ministry of Education, Key Laboratory of Contemporary Anthropology, School of Life Sciences and Institutes of Biomedical Sciences, Fudan University, Shanghai, 200433, China. Tel: +86 21 65642800; Fax: +86 21 55663714; E-mail: ljin007@gmail.com or yanshi@picb.ac.cn

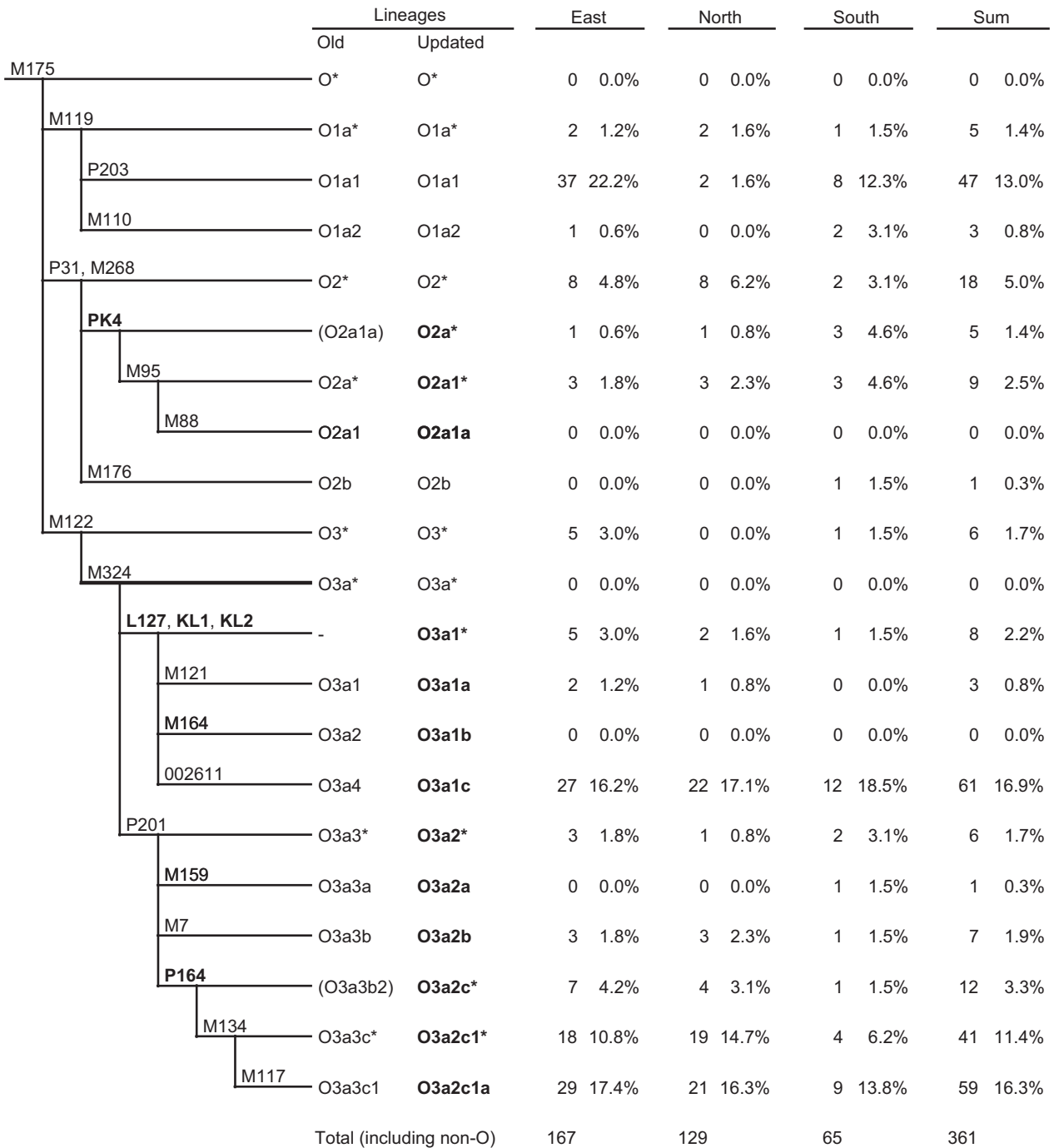
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**Table 1** Primers designed for newly typed SNPs

SNP	RefSNP ID	Y-position (hg18)	Forward primer (5' → 3')	Reverse primer (5' → 3')	Mutation
L127.1	rs17269396	12798981	ttgcatgcagtggtcctcaactcaag	tgaacctggaaggggaacatcac	G > A
KL1	rs17276338	17352235	ttaaatggttactgcaatatttactgt	aatattgccaacatagaagaattgcat	G > T
KL2	rs17323322	21156174	taaatatttctgcaggcttgggca	atgaaaaacatcgtaactatgtatcttc	T > C

Abbreviation: SNP, single-nucleotide polymorphism.

**Figure 1** The updated tree of Haplogroup O and the frequencies of its subgroups in Han Chinese population. The newly located SNPs and renamed lineages are labeled in bold. Samples with derived allele of M164 from our previous studies were typed at L127, KL1, and KL2 in order to locate it in the revised tree. 'East' refers to the samples whose origins are from the provinces of Jiangsu, Anhui, Zhejiang, and Shanghai, whereas 'North' and 'South' refers to the other provinces of which the capitals locate northern or southern to the Line of Qinling Mountains-Huai River, respectively. The samples typed besides Haplogroup O include C-M130 (27, 7.5%), D-M174 (7, 1.9%), G-M201 (1, 0.3%), N-M231 (18, 5.0%), Q-M242 (12, 3.3%), and R-M207 (4, 1.1%).

is corrected and placed upstream of M134 and downstream of P201; no samples were found sharing P164 and M7 mutations. P201 defines Haplogroup O3a2. The relative position of M300 and M333 to P201, 002611, and L127/KL1/KL2 were not determined in this study, because of lack of positive samples as in the previous study,<sup>16</sup> and the relevant lineages can be provisionally named O3a3 and O3a4, respectively. All the samples surveyed that are derivative for M95 have PK4 mutation, whereas there are also (M95–, PK4+) samples indicating that PK4 should be placed upstream of M95, forming an ancient clade in Haplogroup O2.

## DISCUSSION

This study shows that L127/KL1/KL2 and P164 are highly informative for separating a substantial part of O3a-M324 samples in China. O3a1-L127 and O3a2-P201 now appear as two main constituent parts of Chinese population (~20 and ~35%, respectively), whereas individuals belonging to the newly defined paragroup O3a\*-M324(xL127,P201) were not found in this study. This may indicate two main origins of early Han Chinese people, although the exact history can only be discovered through investigation of more populations throughout eastern Eurasia. Although the distribution and age of several clades inside O3a2-P201 (O3a2c1-M134, O3a2c1a-M117, and O3a2b-M7) have been discussed in several studies,<sup>5</sup> few were carried out on O3a1-L127. There are no studies about the origin and date of O3a1c-002611 yet, despite its great prevalence and huge population. Concerning the frequency of O3a2\*-P201(xM134, M7) in Austronesian-speaking populations (up to 55%),<sup>4</sup> it will be interesting to type P164 and M159 on those samples in order to clarify their relationship with the continental populations.

The mutation PK4 separated the former paragroup O2\*-M268,P31(xM95,M176) (6.4% in this study) into two parts: PK4– samples are more abundant in Northern and Eastern parts of China, whereas the PK4+ samples are more frequent in the South, showing the same trend with O2a1-M95.<sup>5,17</sup> Previously, the PK4+ samples were only reported in Pakistan, Nepal, and India from two studies,<sup>14,18</sup> in which no (M95+, PK4–) samples were found. Therefore, the relocation of PK4 in this study does not conflict with the result from those two studies. Considering the amount of O2\*-M268,P31(xPK4, M176) (5% in this study) and the existence of O2a\*-PK4(xM95) samples in China, as well as the discontinuous distribution pattern of O2a1-M95 (frequent in South China, Southeast Asia, and South Asia) and O2b-M176 (abundant in Korea and Japan),<sup>7,9</sup> we may suggest that China was the expansion origin of Haplogroup O2-M268.

With the help of the improved resolution, the newly located SNPs need to be routinely genotyped for the Y-chromosome studies of the East and Southeast Asian populations. The upgraded phylogenetic tree will help to unveil the early history and fine structure of the Asian and Oceanian peoples.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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