Assessment of the forensic application of 50 Y-STR markers in a large pedigree

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ABSTRACT

Short tandem repeats on the Y chromosome (Y-STRs), characterized by paternal inheritance, are valuable in forensic practice. Notably, the potential application of Y-STRs in pedigrees should be drawn upon, especially in China’s surname-concentrated natural villages. The study focused on 50 Y-STRs, including 13 rapidly mutating (RM) Y-STRs that largely constitute the current Y-STR commercial kits, and determined the differences in these Y-STRs between branches in a large pedigree and the discriminatory power of these haplotypes in different units for male relatives. As indicated in the results, 14 inconsistencies were observed at 9 Y-STRs between 10 father-son pairs. In addition, these 50 Y-STR haplotypes discriminated 10 out of 47 father-son pairs, 106 of 148 cousin pairs, 70 of 119 uncle-nephew pairs, 17 of 39 brother pairs, and 14 out of 33 grandfather-grandson pairs in a large pedigree. The RM Y-STR set is able to differentiate close male relatives in a large pedigree.

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Introduction

With the genetic characteristics of paternal inheritance and lack of recombination, Y-chromosomal short tandem repeats (Y-STRs) are extremely useful tools in forensic practice [1]. In particular, they allow the unambiguous detection of male DNA components in mixtures with a high female background, as found in sexual assault or homicide cases. A falsely convicted male whose Y-STR profile does not match that of the sample from a crime scene is excluded [2, 3]. Other suspects or patrilineal relatives of the suspect can be identified by a Y-STR database search. Subsequently, when Y-STRs are combined with autosomal STRs, the perpetrator can be determined. This approach led to the apprehension of a serial killer in northwest China after 28 years [4]. In fact, during the review process of this manuscript, another 28-year-old Nanjing homicide case was solved based on the above technologies.

Plenty of China’s natural villages are surname-concentrated, in which thousands of residents with the same surname share paternal genetic ancestry and are grouped into numerous branches. A village has been reported where the residents have a single surname for dozens of generations. If a crime occurs in these places and the criminal happens to be a member of the patrilineal line, the power of conventionally used Y-STRs is insufficient to exclude numerous patrilineal relatives of the suspect. It would be time consuming to collect samples from thousands of male suspects. Recently, 13 rapidly mutating (RM) Y-STRs have been identified [5], and their ability to differentiate male lineages has been demonstrated [6, 7]. Some of them have been added to recently developed commercial kits, such as the Yfiler® Plus PCR Amplification Kit (Thermo Fisher Scientific, Waltham, MA, USA).

This study focused on 50 Y-STRs including RM STRs, found in the most common Y-STR commercial kits, to observe the variation in Y-STRs in a large pedigree, which will provide an advantage compared with simple mutation investigation.

Materials and methods

Samples and DNA extraction

The samples were 53 male individuals from the four-generation pedigree, and they belonged to five branches designated A, B, C, D and E, as presented in Figure 1. Since the common ancestor had passed away long ago, three live individuals in the first generation were genotyped for 39 autosomal STRs (data
not shown). The kinships between each pair were considered to be full siblings according to the biological full sibling identification code for practice in China [8].

Their bloodstains were collected on FTA Cards (Changchun Bokun Biotech CO., Ltd., Changchun, China). DNA was extracted by using Chelex 100 in a total volume of 100 μL without subsequent quantitation of the DNA amount [9]. Written informed consent was obtained from all study participants in accordance with the Humane and Ethical Research Principles of Sichuan University, and the study was approved by the Medical Ethics Committee of Sichuan University (Ethical approval number: K2019018).

**Y-STR genotyping**

Thirty-seven conventional Y-STRs (DYS393, DYS446, DYS456, DYS522, DYS443, DYS520, DYS458, DYS481, DYS531, DYS19, DYS552, DYS391, DYS635, DYS437, DYS439, DYS389/I/II, DYS388, DYS438, DYS447, DYS390, DYS510, DYS643, DYS587, DYS622, DYS533, Y_GATA_A10, Y_GATA_H4, DYS444, DYS385, DYS460, DYS630, DYS549, DYS392, DYS557, DYS448, DYS527, and DYS459) and 13 RM Y-STRs (DYF387S1, DYF399S1, DYF403S1a/b, DYF404S1, DYS449, DYS518, DYS526a/b, DYS547, DYS570, DYS576, DYS612, DYS626, and DYS627) were involved. Among them, one had “I” and “II” parts (i.e. DYS389) and two had “a” and “b” parts (i.e. DYS526 and DYF403S1). With the primer sequences attained from published literature, their exact map positions were retrieved adopting the UCSC in silico PCR tool (http://www.genome.ucsc.edu/cgi-bin/hgPcr).

These PCR units were amplified with four Y-STR multiplex PCR assays. Three of these multiplexes are commercially available, i.e. the AGCU Database Y24 STR Kit (Database Y24) [10], the AGCU Y SUPP Kit [11] (Y SUPP, AGCU ScienTech Incorporation, Wuxi, China) and the Yfiler® Plus Kit. The RM Y-STR set was designed according to Alghafri’s report [12]. PCR amplification was performed according to the manufacturer’s instructions or those provided by the corresponding reference.

PCR products were separated by multicapillary electrophoresis on the ABI Prism 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The fragment sizes were analysed using Gene-Mapper v.3.2. Allele designations were determined by comparing the sample fragments with those of allelic ladders from the kits or a ladder of RM Y-STRs made in our laboratory. The nomenclature adopted was that of the latest recommendations.
from the DNA Commission of the International Society of Forensic Genetics. RM Y-STR markers were named following the allele designation method adopted by Ballantyne [13].

**Quality control**

For the Database Y24, Y SUPP, and RM set, 9948 was used as control DNA, while 007 was employed for Y-filer® Plus.

**Statistical analysis**

The mutation events of 50 Y-STRs were observed using father-son pairs, and the mutant allele was evaluated for its potential application to distinguish among different branches in a pedigree. The 50 Y-STR markers were selected to constitute different units, including the minimum YHRD marker set, the various commercial kits (PowerPlex Y, PowerPlex Y23, Yfiler, Yfiler® Plus, Database Y24, Y SUPP), and the 13 RM Y-STR sets. For all 50 Y-STRs and each unit, the value was ascertained for differentiating the pairs of father-son, uncle-nephew, brothers, cousins, and grandfather-grandson.

**Results and discussion**

The physical locations of the 50 Y-STR markers on the Y chromosome involved in this work are presented in Supplementary Figure 1. Seven of them are the Y chromosome involved in this work are pre-

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the perpetrator or whether different but related men were involved. The subsequent analysis of the RM Y-STRs will provide further evidence for the exclusion of close male relatives.

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Authors’ contributions

Yi Ye performed the data analyses and wrote the manuscript. Yuran An assisted in the sample collection. Yiwen Yang helped perform the analysis. Hao Wu performed the experiment. Yuzi Zheng contributed to the sample collection. Linchuan Liao contributed to the conception of the study.

Compliance with ethical standards

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Medical Ethics Committee of Sichuan University (Ethical approval number: K2019018) and with the 1964 Helsinki Declaration and its later amendments. Written informed consent was obtained from all individual participants involved in the study.

Disclosure statement

No potential conflict of interest was reported by the authors.

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