Forensic Science International: Genetics Supplement Series 7 (2019) 109–111

Contents lists available at ScienceDirect

Forensic Science International: Genetics Supplement Series

journal homepage: www.elsevier.com/locate/fsigss

Sequence characterization of microvariant alleles at DYS627 and DYS458

Min Lang, Feng Song, Yi Ye, Mingkun Xie, Hong Zhu, Zheng Wang, Yiping Hou*

Institute of Forensic Medicine, West China School of Basic Sciences & Forensic Medicine, Sichuan University, Chengdu, 610041, China

ARTICLE INFO

Keywords:
Y-chromosome
Variant allele
DYS627
DYS458

ABSTRACT

Y chromosome short tandem repeats (Y-STRs) have been widely used in genetic applications and forensic casework. Recently, we found two intermediate alleles, the DYS627 allele 24.1 and the DYS458 allele 15.3, from Chinese Han population. The two allelic variants have not been recorded by the YHRD database. We have examined the molecular structure of these allelic variants by Sanger sequencing. The results showed that this intermediate allele at DYS627 was confirmed as 24.1, the sequence of which showed a base "A" insertion in the 13th repeat unit, and the intermediate allele at DYS458 was confirmed as 15.3, the sequence of which showed a base "G" deletion in the 12th repeat unit. This may be important for individual identification and paternal kinship testing. Besides, more allelic variants detected can be enriched in the Y-STR database.

1. Introduction

Human Y chromosome short tandem repeats (Y-STRs) markers have useful properties for genetic applications, such as historical investigations, genealogical research and forensic evidence examination [1]. The paternal inheritance pattern also confers benefits on Y-STRs in male/female DNA mixtures analysis, kinship analysis, and ancestral origin studies [2,3]. For Y-STRs typing, all forensic laboratories routinely use commercial kits and software. In rare cases, off-ladder (OL) alleles like intermediate-sized variants occur. These allelic variants appear most abundantly in public Y-chromosome databases. While rare overall, their informative frequencies make them attractive for individual identification and paternal kinship testing. Recently, we found two intermediate alleles at DYS627 and DYS458 from Chinese Han population and have examined the molecular structure of these allelic variants by Sanger sequencing.

2. Materials and methods

We have examined one sample defined as intermediate allele at DYS627 locus and one sample at DYS458 locus in our population studies previously performed with 1269 Chinese Han samples [4]. Yfiler Plus PCR Amplification Kit (Thermo Fisher Scientific) was used to simultaneous amplify Y-STR loci according to manufacturer's instruction.

The sequences of primers synthetized for Sanger sequencing of DYS458 and DYS627 were selected from a research article of Ballantyne et al [5]. The sequencing primers were following:

DYS458 F 5′- GCAACAGGAATGAAACTCCAAT -3′

DYS458 R 5′- GTTCTGGCATTACAAGCATGAG -3′

DYS627 F 5′- CTAGGTGACAGCGCAGGATT-3′

DYS627 R 5′- GGATAATGAGCAAATGGCAAG- 3′

Singleplex PCRs were performed in a 20 μl final volume containing 10 μl Multiplex Master Mix (Qiagen), 1 μl PCR primer, 1 ng DNA template or control DNA. PCRs were performed on a ProFlex 96-well PCR System (Thermo Fisher Scientific). Thermal cycling consisted of: enzyme activation of 10 min at 95 °C; followed by 10 cycles of 30 s at 94 °C, 30 s at 60 °C touch-down, 45 s at 72 °C and 25 cycles of 30 s at 94 °C, 30 s at 50 °C, 45 s at 72 °C; final extension of 45 min at 60 °C and hold at 4 °C until removal from the thermocycler. After amplification, the products were detected by sequencing (Tsingke Biological Technology, China).

3. Results and discussion

In our population studies, a total of two samples demonstrated alleles that were "OL" in the process of Y-STR genotyping, one sample at DYS458 (Fig. 1A) and the other one at DYS627 (Fig. 1B). They were confirmed by reamplification using the same condition as before. To determine molecular structure characterization of these two intermediate alleles, we have examined the allelic variants by sequence analyses. After bidirectional sequencing, the intermediate allele at DYS458 was confirmed as 15.3, the sequence of which showed a base "G" deletion in the 12th repeat unit (Fig. 2A), and the intermediate allele at DYS627 was confirmed as 24.1, the sequence of which showed a base "A" insertion in the 13th repeat unit (Fig. 2B). The two intermediate alleles both have not been recorded by the YHRD database and

* Corresponding author at: 3-17 Renmin South Road, Chengdu, 610041, China.
E-mail address: forensic@scu.edu.cn (Y. Hou).

https://doi.org/10.1016/j.fsigss.2019.09.043
Received 27 August 2019; Accepted 23 September 2019
Available online 05 October 2019

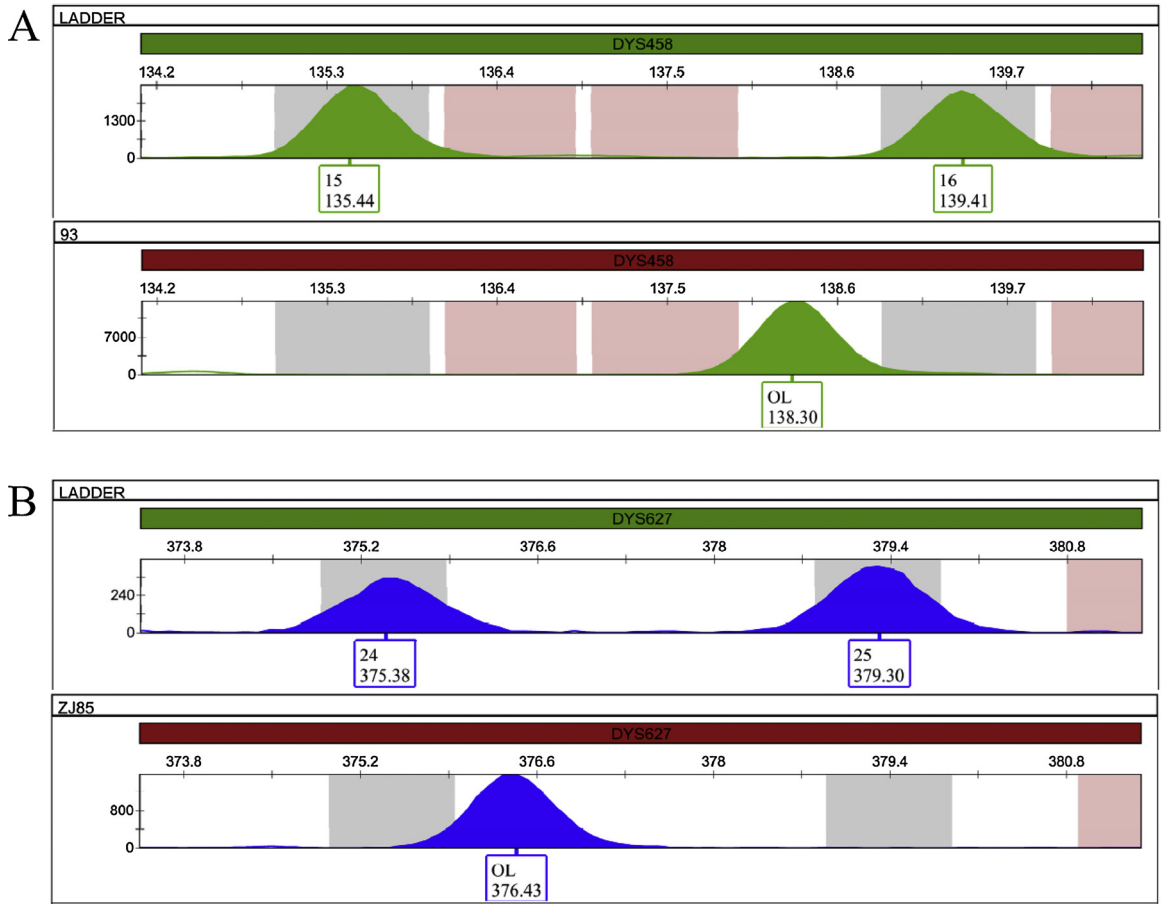

Fig. 1. Genotypes of allelic ladder and two samples labeled as "OL". Fig. 1A shows the sample (93) is labeled as "OL" at DYS458 and Fig. 1B shows the sample (ZJ85) is labeled as "OL" at DYS627.

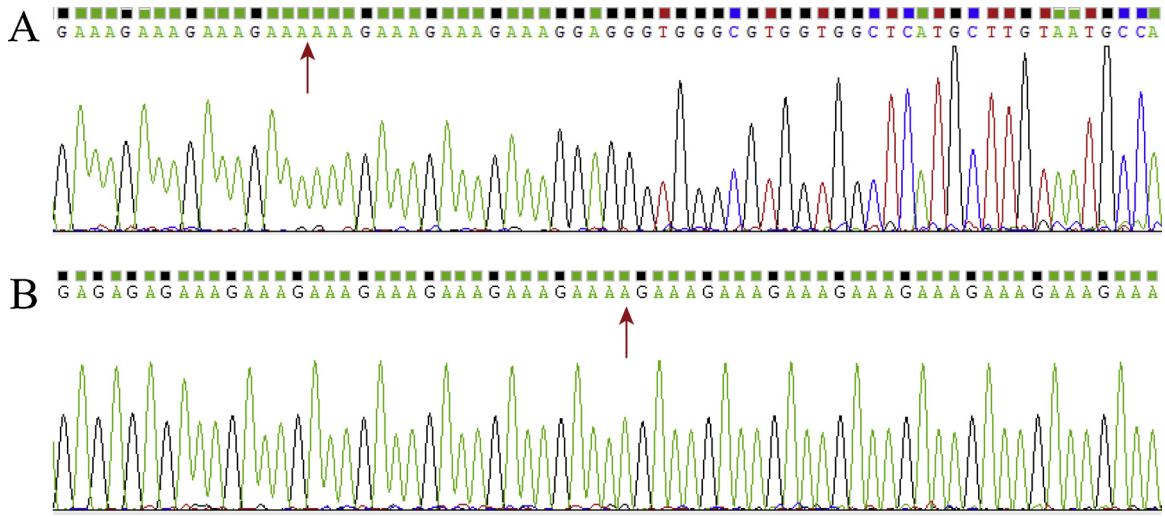

Fig. 2. Sequencing results of intermediate allele at DYS458 and DYS627. Fig. 2A shows the results of DYS458 and the red arrow and the line indicate the 12th repeat unit where one base "G" is lost. Fig. 2B shows the results of DYS627 and the blue arrow and the line indicate the 13th repeat unit where one base "A" is inserted. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

can be enriched in the Y-STR database.

Recently, publicly accessible online databases consisting of Y-STR haplotypes have grown large enough to detect these allelic variants. Previous analyses have also found plenty of intermediate alleles at another Y-STR locus, such as indicated as 0.2 at DYS458 [6]. These rare alleles were mainly caused by deletion or insertion of individual bases in allelic fragments. With low frequencies in population, these variant alleles are benefit to individual identification and paternal kinship testing. Besides, some analysis found that the molecular characterization of such variant alleles had a closely association to some a defined Y chromosome branch and these alleles in combination with haplogroup-defining markers might expose new phylogenetic substructure within the Y chromosome haplogroup tree [7].

4. Conclusion

In this study, a total of two intermediate alleles, the DYS458 allele 15.3 and the DYS627 allele 24.1 observed in the process of Y-STR genotyping. Both of them have not been recorded by the YHRD database. The molecular structures of these two allelic variants are further verified by Sanger sequencing. More variant alleles detected can be enriched in the Y-STR database and have great significance in individual identification and paternal kinship testing.

Declaration of Competing Interest

None.

Acknowledgement

This study was supported by grant from the National Natural Science Foundation of China (81871532).

References

[1] M. Kayser, Forensic use of Y-chromosome DNA: a general overview, Hum. Genet. 136 (2017) 621–635.
[2] M. Prinz, K. Boll, H. Baum, et al., Multiplexing of Y chromosome specific STRs and performance for mixed samples, Forensic Sci. Int. 85 (1997) 209–218.
[3] A. Seman, A.M. Sapawi, M.Z. Salleh, Towards development of clustering applications for large-scale comparative genotyping and kinship analysis using Y-short tandem repeats, OMICS 19 (2015) 361–367.
[4] M. Lang, H. Liu, F. Song, et al., Forensic characteristics and genetic analysis of both 27 Y-STRs and 143 Y-SNPs in Eastern Han Chinese population, Forensic Sci. Int. Genet. 26 (2019) 13–20.
[5] K.N. Ballantyne, M. Goedbloed, R. Fang, et al., Mutability of Y-chromosomal microsatellites: rates, characteristics, molecular bases, and forensic implications, Am. J. Hum. Genet. 87 (2010) 341–353.
[6] G. Iacovacci, E. D'Atanasio, O. Marini, et al., Forensic data and microvariant sequence characterization of 27 Y-STR loci analyzed in four Eastern African countries, Forensic Sci. Int. Genet. 27 (2017) 123–131.
[7] N.M. Myres, K.H. Ritchie, A.A. Lin, et al., Y-chromosome short tandem repeat intermediate variant alleles DYS392.2, DYS449.2, and DYS385.2 delineate new phylogenetic substructure in human Y-chromosome haplogroup tree, Croat. Med. J. 50 (2009) 239–249.