Research Article

An Application of ITO Analysis in Secondary Kinship Identification

Peng Zhou,1,2 Xuemei Chen,1 Qianrong Wang,3 Junfu Wang,1,2 Xiaoqun Xu,1,2 and Ran Wei1,2

1Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan, China
2School of Clinical and Basic Medical Sciences, Shandong First Medical University & Shandong Academy of Medical Sciences, Jinan, China
3The Third Affiliated Hospital of Shandong First Medical University, Jinan, China

Correspondence should be addressed to Xiaoqun Xu; xuxiaoqun@sdfmu.edu.cn and Ran Wei; weir_vian@sina.cn

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Objective. As the methods of the paternity and kinship testing have been developed, the second-degree and more distant relationships remain challenging in forensic science. Currently, the ITO method is the mainstream method to clarify the kinship between two individuals.

Methods. In this study, the ITO algorithm was used to calculate the uncle-nephew index based on 55 autosomal short tandem repeats (STRs) loci that were universally used for forensic identification. 19 STRs loci in Y chromosome were used for verification of the kinship.

Results. The cumulative uncle-nephew index between A and B was calculated to 0.993 by the analysis of the genotyping results of 21 STRs. When genotyping results of the other 34 STRs were added to the calculation algorithm, the cumulative uncle-nephew index between A and B was promoted to 227.928. Meanwhile, genotyping results of 17 Y-STRs loci showed that A and B shared the same Y-STRs haplotype that was in accord with the paternal inheritance law.

Conclusion. The biological uncle-nephew relationship between A and B are identified by applying the statistical principles and genetic technologies.

1. Introduction

In forensic genetics, short tandem repeats (STRs) on autosomes is the frequently-used genetic markers in the mainstream at present [1, 2]. The stochastic ITO transition matrices provided by Li and Sacks in 1954 is a traditional methods to obtain the joint STR genotype distribution and genotypic correlations between any specified pair of non-inbred relatives [3, 4]. However, the sibling identification is complicated, and the conclusions risk uncertainties in some cases, because the potential intimate kinship between the two individuals involved in the kinship testing may limit the amount of genetic information that is usable for the identification. In this case study, an adult male (A) suspected that he might be abducted and trafficked into his current family at his early age. Because both the suspected father and the suspected mother died due to physical reasons and no material samples could be collected, the paternity identification could not be conducted. So the man (A) requested an identification of the uncle-nephew kinship with his alive suspicious uncle (B). We applied the ITO algorithm using STRs in autosomes and Y chromosome in the identification of this secondary kinship. The validity of the ITO algorithm will provide more reliable evidences for the kinship identification.

2. Materials and Methods

2.1. Sample Collection and DNA Extraction. Blood spot samples were taken from the fingertip of A and B. DNA samples were extracted by 5% Chelex-100 (Sigma-Aldrich, USA) extraction method.

2.2. PCR Amplification. 55 pairs of primers for STRs on autosomes were obtained from SiFaSTRTM 23 Plex Identification System (China Academy of Forensic Science, China),
GoldeneyeTM 22NC Identification System (Jidian Cognitive Technology Co., Ltd., Beijing, China), and AGCU 21+1 STR Fluorescence Detection kit (Zhongde Meilian Biotechnology Co., Ltd., Wuxi, China). 17 pairs of primers for Y STRs were obtained from Goldeneye DNA 27Y Identification System (Jidian Cognitive Technology Co., Ltd., Beijing, China). PCR reactions were performed according to the manual of each kit.

2.3. Capillary Electrophoresis and Genotyping Analysis. Capillary electrophoresis was performed using a 3130 XL Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA, USA), and genotype detection and analysis were performed using GeneMapper ID-X software (Thermo Fisher Scientific, Waltham, MA, USA).

2.4. Statistical Analysis. The ITO method was applied to calculate the uncle-nephew index using genotyping data of 55 autosomal STRs loci, and the verification of the uncle-nephew kinship is carried using genotyping data of 17 Y-STRs loci.

3. Results

3.1. Genotyping of Autosomal STRs Loci. With the credible negative and positive quality controls, valid genotyping of total 55 STRs loci on autosomes in A and B was conducted. The genotyping results are shown in Table 1. A and B had different STRs on homologous chromosomes at 14 loci (vWA, D21S11, D18S51, FGA, D15S659, D3S3045, D17S1290, D12S391, D6S1043, D7S820, Penta D, TH01, vWA, D7S3048, D5S2500, and D2S1776). At the other 41 loci, A and B shared at least one STR haploid. Among them, at 7 loci (D19S433, D13S317, D4S2366, D13S325, D6S474, D6S1017, and D9S1122), A and B shared the same genotype.

3.2. Genotyping of Y-STRs Loci. With the credible negative and positive quality controls, valid genotyping of total 17 Y-STRs loci in A and B was conducted. The genotyping results are shown in Table 2. A and B shared the same genotype in all the 17 Y-STRs loci.

3.3. Calculation for the Uncle-Nephew Index. The uncle-nephew index is calculated using the formula $W = PI / (PI + 1)$. The cumulative uncle-nephew index between A and B was calculated to 0.993 by the analysis of the genotyping results of 21 STRs (from SiFaSTRTM 23 plex identification...
system). This value was in the middle area that the uncle-nephew kinship could not be identified or excluded. When genotyping results of the other 34 STRs (from Goldeneye™ 22NC Identification System and AGCU 21+1 STR Fluorescence Detection kit) were added to the calculation algorithm [5], the cumulative uncle-nephew index between A and B was promoted to 227.928. The uncle-nephew index between individuals A and B was calculated as 21%, 40%, 69%, and 95% based on the genotyping data of 19, 29, 39, and 55 autosomal STRs separately. Meanwhile, genotyping results of 17 Y-STRs loci showed that A and B shared the same Y-STRs haplotype that was in accord with the paternal inheritance law. Therefore, based on the existing information and genetic analysis results, there is a high possibility that a biological uncle-nephew relationship between A and B exists.

### 4. Discussion

A complete description of the degree of relatedness of two individuals is a common and fundamental request in the forensic genetics [6]. In this case, there is an urgent demand for kinship identification without the genetic information of parents. However, there is no common standard for uncle-nephew kinship identification. So firstly, we used the genotyping results of 21 STRs from the conventional kit, SiFaSTRTM 23 plex identification system. The calculation method was revised according to the preliminary result, and the sufficient number of STRs loci was added. All STRs loci used in this study are all loci frequently used in forensic identification practice and have abundant population data which provide a reliable basis for the calculation reliability and the implementation feasibility. Meanwhile, when the uncle-nephew kinship was identified with relatively low index, the Y-STRs locus is a useful supplement in case that the tested individuals are all male [7]. The same Y-STRs haplotype found in the tested individuals enhance the reliability of the identification.

This study calculated the uncle-nephew index using ITO method [3]. The ITO method is a classic way to identify the kinship between two individuals. Shao et al. pointed out that after the conclusion that shared alleles cannot be excluded from the analysis, ITO method can be further used to establish discriminant assumptions according to the specific case to obtain objective and reliable identification opinions [8]. In our study, it is demonstrated that when more autosomal STRs were used, the more uncle-nephew index between individuals A and B was obtained. It is demonstrated that the accuracy rate of the uncle-nephew kinship identification between two individuals increases with the number of the genetic markers. Therefore, more loci should be genotyped within the maximum testing capacity of the forensic laboratory’s capacity for the complex kinship identifications, such as uncle-nephew relationships. However, the sufficient number of genetic marker loci and the rage of the probability value to verify the kinship in the complex cases should be investigated.

With the development of high-throughput sequencing and the establishment of genome database, kinship testing based on multiple genetic markers, such as SNPs and micro-haplotypes, has great valuable practical applications [9, 10]. In the future, we will apply more genetic markers, such as SNPs and indels, in solving complex kinship testing problems based on high-throughput sequencing [11, 12].

### Data Availability

The experimental data used to support the findings of this study are available from the corresponding author upon request.

### Conflicts of Interest

The authors declared that they have no conflicts of interest regarding this work.

### References


### Table 2: Genotyping of 17 Y-STRs loci in A and B.

<table>
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<th>Y-STRs loci</th>
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