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Original communication

A grey zone approach for evaluation of 15 short tandem repeat loci in sibship analysis: A pilot study in Indian subjects

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Abstract

The evaluation of 15 STR loci Applied Biosystems Identifiler kit for sibship determination in Indian subjects is reported. Cumulative sibship indices (CSIs) calculated following standard methods in sibling pairs and non-sibling pairs, showed mean values comparable to other reports. Mean CSI value in sibling group was higher than in corresponding non-sibling group. Moderately high value of CSI in one of the non-sibling pairs and a very low likelihood ratio favoring non-relatedness in a known sibling pair did not allow binary decision about sibship status. To deal with this problem a grey zone approach has been applied to sibship test. It is concluded that the 15 loci STR kit can be reliably used for inferring sibship between pairs of individuals by defining a grey zone of a sibship test as an area of likelihood ratio values where the discriminatory performance is insufficient.

Keywords: Forensic science; Sibship; STR loci; Grey Zone; Human identification; Cumulative sibling index

1. Introduction

Forensic DNA analysis is traditionally a science of comparison. In cases of personal identification of unknown human remains, a direct comparison can be carried out between the DNA profiles developed from the remains with those obtained from the personal articles of the deceased.[1] While direct comparison provides the most meaningful conclusion, a practical problem with the use of personal articles as references is that these articles may be unavailable for study or they may not yield sufficient DNA for analysis. Hence the Forensic DNA analysts mainly rely on reference samples of the close biological relatives of the deceased for indirect comparison. Parents of the deceased in the absence of mutation, offer a rich source of genetic information for inferring correct relationship.

In instances where parentage analysis is not feasible the investigators turn to the next closest genetic kinship, i.e., of the full siblings, where allele sharing by decent is an observed event. Sibship indices are used in determining a hypothesized relationship between two persons.[2] This approach uses a likelihood ratio to evaluate the support of the evidence under the hypothesis that the evidence profile is from the sibling or from an unrelated person.

STR multiplexes assays are now the dominant forensic human identification technology.[3] A core set of 15 STR markers are now being used worldwide and due to high discrimination power are supposed to maximize the relative probabilities of allele sharing by descent versus allele sharing by chance but their accuracy in discriminating siblings from non-siblings is unclear. Previous studies using various STR locus combinations reflect that it is difficult to determine exactly how many loci are necessary to achieve a certain level of power in sibship inferences and highly impossible to provide a universal CSI cut-off point for

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demarcating siblings from unrelated group.[4,5] These studies also suggest for creation of an initial reference standard in every DNA laboratory on which the decision to apply the test convincingly can be taken. The present case-control sibship study in Indian subjects was designed with an aim of generating suitable values of CSI using the 15 panel STR multiplex systems. We have examined the role of CSI in predicting sibship in Indian subjects across the guidelines established for degree of certainty based on the calculated LR values.[6] We determined conventional sensitivity and specificity, as well as positive and negative likelihood ratios (LR+ and LR−). This has allowed us for defining the grey zone of sibship test for screening discrimination.

2. Materials and methods

In a pilot study, we selected 33 unrelated volunteer family units, consisting of a mother, father and two siblings. 33 pairs of unrelated non-siblings were taken for control studies. The whole blood samples were archived on FTA cards (Whatman Biosciences® UK).

2.1. DNA purification

The DNA purification steps were performed as per the manufacturer's instructions (Whatman Biosciences® UK).

2.2. DNA amplification

AmpFlSTR identifiler™ multiplex assay (Applied Biosystems, Foster City, California) for 15 autosomal, codominant and unlinked STR loci was used for the present study. The amplification reactions were performed according to the manufacturer's instructions. The samples were amplified in a 2400 thermal cycler (PE Applied Biosystems, Foster City, California).

2.3. Separation and detection of amplified products

Amplified product (1.5 μl) was dispensed into a tube containing 24 μl of Hidi Formamide (Applied Biosystems, Warrington, UK) and 0.5 μl of Liz-500 size standard (Applied Biosystems, Warrington, UK). The tube was heated to 95 °C for 3 min and then snap chilled in a portable chiller for 3 min. Electrophoresis was carried out on an ABI 310 GeneticAnalyzer (Applied Biosystems, Foster City, California) with GeneScan 2.1 software. Genotypes were determined by comparing the size of the unknown fragment to the allelic ladder that was run in parallel.

2.4. Statistics and evaluation

Once the genotypes for the 33 family units were determined, we conducted paternity tests to establish that the two children of each family unit were biological children of both parents. Sibship index values were then investigated in 33 true sibling pairs and compared with those of other 33 non-related random pairs.

Paternity and sibship indices were calculated using 'PATCAN' software.[7] Average power of discrimination values fall within the range of 0.718 and 0.941 for 15 STR loci in our in-house population data. The typical paternity index is 549313, while the minimum cut-point established for determining paternity is 1000. Although no consensus has been reached on CSI cut-off value to use with sibship test, the cut-off level of 1 was used to define a positive test result. Sensitivity and specificity were determined at this level. Test sensitivity was calculated as true positive tests per total sibling pairs tested. Test specificity was calculated as true negative tests per non-sibling pairs tested. Both these parameters were expressed as percentages. Likelihood ratio for positive test result (LR+) and Likelihood ratio for negative test result (LR−) were defined in terms of determined sensitivity and specificity: LR+ = sensitivity/1 − specificity and LR−=1 − sensitivity/specificity. Two cut-off points one associated with the minimal desirable value of LR(+) and the other maximal desirable value of LR(−) were identified delimiting the grey zone (area of inconclusive CSI values) as described by Joël Coste and Jacques Pouchot.[8]

3. Results

The results of cumulative sibship indices (CSIs) for both sibling and random pairs are shown in Fig. 1. The lowest CSI value of 0.0000003 was noted in the non-sibling pair, while the highest value of 116416023 was illustrated in a sibling pair. For sibling pairs both mean and median CSI values were significantly higher when compared to non-sibling pairs. In the sibling group 31 cases (93.94%) had CSI values greater than 1. Two known sibling pairs (6.06%) illustrated CSI < 1 with one of them ranked as low as 0.0019.Thirty cases (90.9%) of the random pairs had CSI values less than one. Fortuitously one non-sibling pair had CSI as high as 17.2. Fig. 2 shows the sensitivity and the specificity of the test at CSI cut-off level of 1. The likelihood ratio indicating the value of the test for increasing certainty about a positive judgment (LR+) is 10.3 (CSI cut-off point = 1). The likelihood ratio indicating the value of the test for increasing certainty about a negative opinion (LR−) is 0.067 (CSI cut-off point = 1). A grey zone keeping under surveillance the inconclusive CSI values for sibship test is shown in Fig. 3.

The distribution of allele sharing for the sibling and the non-siblings is shown in Fig. 4. There were a total of 495 observations (15 loci × 33 pairs) for each group. The sibling pair with lowest CSI value (0.0019) conspicuously did not show the phenomenon of '2- allele sharing' at any of the loci. Zero allele sharing was observed at three loci in this pair. There were nine non-sibling pairs that showed no 2 alleles sharing at any of the loci. There was a perceptible '0-allele sharing' at 12 loci in one of the non-sibling pairs in the midst of the lowest CSI.