



# Male individualization with Rapidly Mutating Y-STRs in deep rooted endogamous pedigrees from Sindhi population of Pakistan



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## Introduction

Y-chromosomal short tandem repeats (Y-STRs) are commonly used to identify males for criminal justice purposes. However, Y-STRs which are generally used in the forensic field have low haplotype diversity in some populations and fail to discriminate between male relatives. A recently identified set of 13 rapidly-mutating (RM) Y-STRs (DYF387S1, DYF399S1, DYF403S1a/b, DYF404S1, DYS449, DYS518, DYS526, DYS547, DYS570, DYS576, DYS612, DYS626, and DYS627) typically reveal higher haplotype diversities than the commercially available Y-STR sets and allow differentiating male relatives for which commercial Y-STR sets are usually not informative. In current study, we analyzed haplotypes of the 13 RM Y-STRs and the PowerPlexY23 loci in 86 individuals from 18 endogamous Sindhi families from Pakistan, from which a total of 216 pairs related by 1-7 generations were investigated.

## Materials and Methods

### Samples

Blood samples were collected from 86 male members of 18 unrelated volunteer Sindhi families residing in the interior parts of Sindh province of Pakistan including Hyderabad and Nawab shah. All participants gave their informed consent orally as well as in writing after we explained the aims and procedures of the study. The study was approved by ethical review committee of University of Health Sciences Lahore, Pakistan. DNA was isolated from blood using ReliaPrep™ Blood gDNA Miniprep System (Promega, Madison, USA) according to the manufacturer's instructions.

### Y-STR genotyping

To determine Y STR haplotypes, the PowerPlex Y23 (Promega) and two in-house multiplex PCR sets for 13 RM Y-STRs developed by Shin *et al.* were used. This in-house multiplex PCR system yields small-sized amplicons (< 400 bp) and allows to separate three copies of DYF403S1a into two copies of DYF403S1a and one copy of DYF403S1b1 with the use of sequence-specific primers. In addition, the other copy, which had been defined as DYF403S1b by Ballantyne *et al.* (Hum Mutat, 2014), was renamed as DYF403S1b2 because it has similar flanking region sequence to DYF403S1b1.

## Results

In current study, we have genotyped 216 pairs of relatives (50 father-son pairs, 6 grandfather-grand son pairs, 63 brother pairs, 34 uncle-son pairs, 25 1<sup>st</sup> cousin pairs, 5 nephew/uncle-son pairs, 7 2<sup>nd</sup> cousin pairs, 14 1<sup>st</sup> cousin once removed and 12 2<sup>nd</sup> cousin once removed). In this dataset, the RM Y-STR set allowed differentiation by at least one allelic difference (i.e. mutation) in at least one locus in 71 (32.87%) of the total 216 pairs related by 1-7 generations, while PowerPlex Y23 allowed 23 (10.23%) and Y-filer only allowed the separation of 9 (4.17%) in 216 these relative pairs. In particular, RM Y-STRs allowed individual differentiation from 16% of father-son pairs, 33% of brother pairs, 68% of 1<sup>st</sup> cousin pairs, 86% 1<sup>st</sup> cousin once removed pairs (5 degrees), 29% of 2<sup>nd</sup> cousin pairs (6 degrees) and 25% of 2<sup>nd</sup> cousin once removed pairs (7 degrees). RM Y-STRs have 22.64% higher differentiation rate when it compared with PowerPlex Y23 loci and 28.70% when compared with Y-filer loci (Fig. 1).

Fig. 1. Male relative differentiation (%) using RM Y-STR, PowerPlex Y23 and Yfiler Y-STR sets

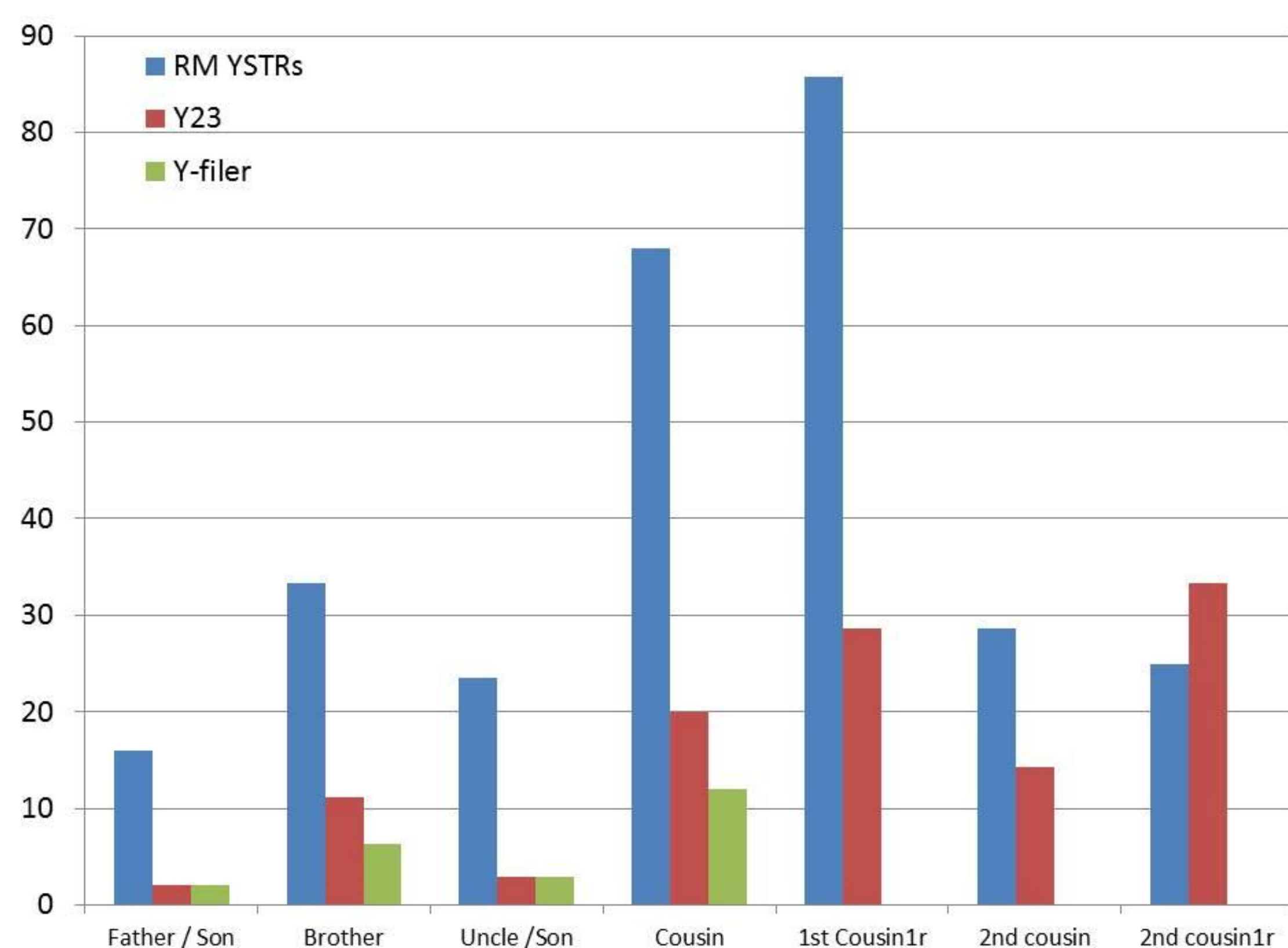
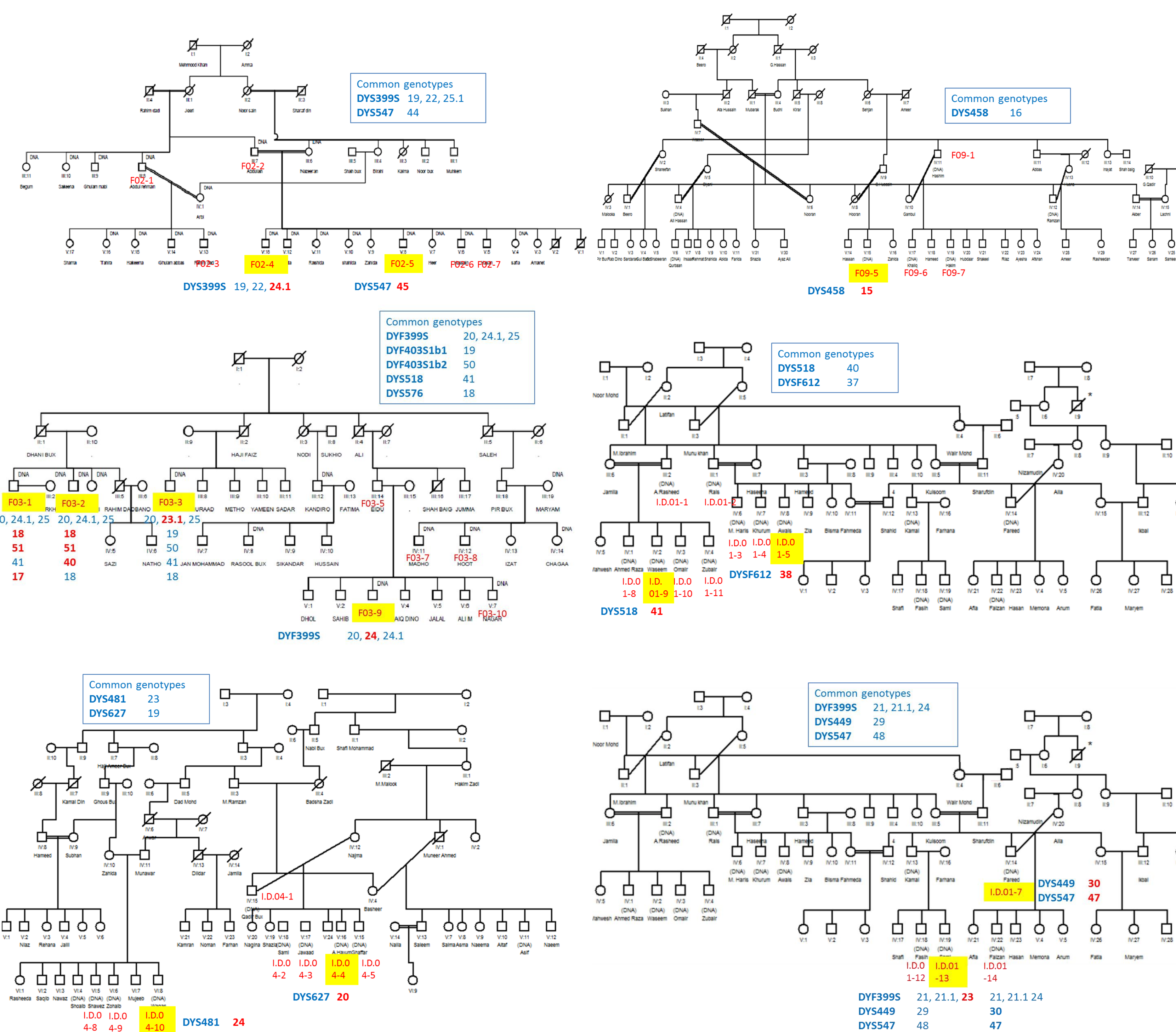


Fig. 2. Pedigrees showing Y-STR mutations in the same paternal lineage. Among 18 endogamous Sindhi families from Pakistan, 11 showed Y-STR mutations and 6 were shown here. Mutations are indicated in red, and males with mutations are highlighted in yellow.



## Conclusion

- Our results follow the findings of previous study (Ballantyne et al. Forensic Sci Int Genet, 2012), which indicated that the RM Y-STR set could considerably increase the discrimination power in paternal relatives as well as in unrelated males.
- RM Y-STR panel was successful in differentiating between closely related males from 18 endogamous Sindhi families.
- However, the differentiating power of RM Y-STRs in Sindhis from Pakistan seemed lower than those previously reported for other closely related males (16% of father-son pairs and 33% of brother pairs vs. nearly 50% of the father-son pairs and 60% of brothers pairs).