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# Analysis of recombination and mutation events for 12 X-Chr STR loci: A collaborative family study of the Italian Speaking Working Group Ge.F.I

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ABSTRACT

According to the ISFG guidelines on the use of X-chromosome, the biostatistical evaluation in kinship analysis is based on a likelihood ratio approach, but since in calculation linkage and recombination events should be accounted for, an accurate estimate of mutation and recombination rates of X-markers analyzed is required. Due to the mode of genetic transmission and physical location, sometimes X-chromosome markers can be more informative than autosomal STRs and their analysis could be considered a supplementary tool in DNA testing.

The increased demand to forensic laboratories for kinship investigations in complex cases explains the need not only to enlarge the Italian population database for 12 X-STRs routinely used for forensic application, but also to investigate the recombination fractions among markers.

A collaborative exercise of the Italian Speaking Working Group Ge.F.I. was organized to evaluate mutation and recombination events in 12 X-STRs included in the Investigator Argus X12 kit. In order to explore the segregation stability, three-generation families (grandpa-mother-son) and two-generation families (mother-sons, father-daughters) for a total of 269 pedigrees were analyzed and calculations to estimate the recombination fractions between pairs of markers and mutation rates were performed. The statistical analysis showed evidence of inter- and intra-cluster recombination events, with almost free recombination for junction markers of linkage groups I and II and reduced recombination for linkage groups III and VI. We observed one- and two-step mutations, with an average value of $2.9 \times 10^{-3}$.

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## 1. Introduction

The present study derived from the increasing requests to forensic labs to solve complex kinship cases especially when the alleged father is not available, and half-sisters are involved. In this specific cases, X-STRs analysis is often the best choice to get more information than autosomal markers. For statistical evaluation, some considerations are required when using chromosome X markers in kinship testing, for the possibility of linkage and linkage disequilibrium (LD). As recommended in [1], likelihood ratio (LR) calculation should consider recombination events for X markers included in a linkage group. Larger is the database, more accurate is the estimate of recombination fraction and more reliable is LR calculation. To date, three generation family studies on the segregation stability in X markers included in the Investigator Argus kit, the most popular validated kit in use for forensic application, are not reported for the Italian population. The aim of the collaborative exercise of the Italian Speaking working group (GeFI) was to evaluate possible mutational and recombination events for X-STR markers in Italian pedigrees.

## 2. Material and methods

Blood and saliva samples were collected from 934 individuals belonging to 269 three and two generation families. Samples have been collected under informed consent of the participants and anonymized. DNA was extracted and typed for the 12 X-STRs included in the Investigator Argus kit (Qiagen). PCR products were separated by capillary electrophoresis in ABI instrument (ABI3500, ABI3130, ABI 310, Applied Biosystems) and analyzed according to the manufacturer instructions including also positive and negative controls.

For linkage analysis, pedigrees were split in two types of informative sub-families. Type I family was composed by a phased mother and one or more of her phased children (grandpa, mother, son); Type II family was composed by a non-phased mother and two or more of her phased children (mothers, daughters and sons). Note that a single pedigree could produce any number of type I and II families (Fig. 1). We considered linkage-informative meiosis when children's recombined maternal haplotype could be inferred, i.e. in sons, and also in daughters when their father is genotyped. Moreover, in type I families, the mother haplotypes are known and thus one child is enough to provide information on recombination, while, in type II families, at least two sons are needed.

To compute the likelihood of each family, i.e. the likelihood that the mother meiosis produced the maternal haplotypes observed in her children, a likelihood-based approach was used as described in [2]. This method takes as input the family genotypes and estimates inter-marker recombination rates for multiple STR loci allowing for possible one-step mutations. All consistent family likelihoods are summarized for having the overall likelihood with the same prior probabilities for all haplotypes constellations because not considering LD within linkage groups.

Likelihood maximization was performed using high-level programming language Python v.3.6 and multiple runs were performed first with 3 different sets of starting values for the inter markers recombination rate and then investigating the effect of increasing values to find the recombination rates that best fit for the observed dataset, i.e., that maximize its likelihood. Mutation analysis was carried out by Microsoft office Excel software.

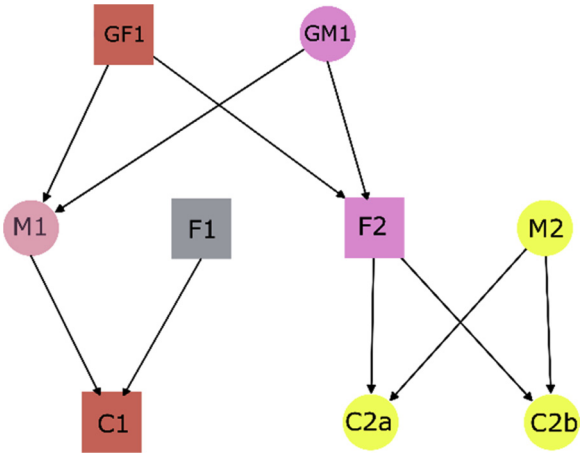


**Fig. 1.** Example of Pedigree AV_BN_10 consisting of one Type I family and two Type II families. Type I : Grandfather GF1, Daughter M1 and Grandson C1; Type II : Grandmother GM1, Son F2, Daughter M1; Type II : Daughter in law M2, Grandaughter C2b, Grandaughter C2b.

## 3. Results

From a total of 269 pedigrees, we extracted 40 families of type I and 147 of type II. The number of informative meiosis was 69 in type I families and from 346 to 353 in type II families (Supplementary TableS1). One daughter from a type II family was excluded since her phasing was ambiguous because a mutation at locus DXS10103. Then the likelihood computation was performed from family genotypes to exclude children with likelihood = 0, i.e. this is the case when a non-one-step mutation is observed, either fractional or greater than one. In this way one from a type I family and 10 children from type II families showed inconsistencies and were excluded from further analysis as well as 5 families of type II, so families of type II dropped to 142 pedigrees (Supplementary TableS2).

From likelihood maximization, the maximum value of recombination fraction was found between linkage groups I and II (0.45), with almost free recombination for junction markers of the two linkage groups, a lower recombination rate (0.30) was observed for linkage groups III and VI (Supplementary TableS3). Moreover, non-negligible recombination fractions were observed also intra cluster for all the four linkage groups, exceeding 1% for DXS10148-DXS10135, DXS10103-HPRTB and DXS10146-DXS10134 marker pairs.

Twenty mutations were found in a cumulative number of 6876 meiosis from 217 father-daughter and mother-son pairs: 18 were single-step mutations and 2 showed one the gain of two repeat units and the other a loss of two repeat units (Supplementary TableS1). DXS10135 locus showed the highest number of mendelian inconsistencies. The overall 12 X markers mutation rate was $2.9 \times 10^{-3}$. Moreover, we observed a triallelic pattern for DXS10079 locus in a father-daughter pair.

## 4. Discussion

X STRs markers show higher efficiency parameters than autosomes and they can be useful for any parent–child relationship that involves at least one female. For closely linked markers it is advisable to consider linkage and linkage disequilibrium for most precise likelihood calculation especially when using these markers in kinship testing. In order to estimate the recombination rate, segregation analysis is performed based on family studies and large maps of genetic distances. A collaborative exercise to investigate the recombination and mutation events of the 12 STRs markers included in the Investigator Argus kit was handled in three and two generation pedigrees from an Italian sample. The recombination fractions were estimated by a likelihood-based method for multiple STRs markers from family genotypes. When likelihood is zero, inconsistencies due to non-one-step mutation are observed, emerging possible typing errors in the dataset. Multiple runs were performed with different sets of values of recombination rates investigating the effect of increasing values; all runs produced very similar recombination rate estimates, proving the trustworthiness of the method. Comparing to Nothnagel et al. [2], our data agree with findings reported in the study, even if we observed slightly lower values of

recombination fractions between all the three junction markers of linkage groups, though values fall in 95% support intervals (Supplementary TableS4). The average mutation rates estimated for the 12 X-STRs markers is similar to previous reports [3,4].

## 5. Conclusion

The present study on linkage analysis in Italian pedigrees reflects the findings of previously studies and points out the presence of intra-cluster recombination which should be considered for statistical evaluation in kinship testing. Likelihood maximization is a useful approach for estimation of recombination rate and to detect inconsistencies also due to typing errors. However, it could be useful to implement the function by including also the probability of two-step mutations as we observed in this study.

## Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.fsigss.2019.10.027.

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