



## Letter to the Editor

**Genetic characterization of ten X-STRs in a population from the Spanish Levant**

Dear Editor,

X chromosome short-tandem repeats (X-STR) assays are helpful in complex kinship testing cases where autosomal and Y chromosome analyses convey little information. Their application has distinct advantages in mother-son and father-daughter kinship testing. Paternal relatives can be tested even in cases of deficiency paternity [1].

The purpose of this study was to create an allele frequency database for a southeast population in Spain and forensic efficiency parameters using X-linked chromosome STR markers [2]. This is the first report regarding the ten panel of X-linked chromosome markers developed by the Spanish and Portuguese Speaking Working Group of the International Society for Forensic Genetics (GHEP-ISFG) applied to the characterization of a southern population of the Iberian Peninsula. The Region of Murcia is located in the southeast coast of Spain within the Spanish Levant. The Spanish Levant is the collective name for four Mediterranean provinces on the Mediterranean coast.

A sample of 172 unrelated individuals was selected for this study from the Murcia Twin Registry (MTR) database at the University of Murcia [3]. All the donors chosen for this study are residents of the Region of Murcia. Peripheral blood samples were collected and provided by the MTR following ethical requirements stipulated in governing laws [4,5]. Samples were extracted following standardized protocols at the National DNA Bank from the University of Salamanca. DNA quantitation was conducted at the BIOMICs Research Group from the University of the Basque Country using Quant-iT PicoGreen<sup>®</sup> dsDNA Assay Kit (Invitrogen, Carlsbad, California) on a DTX880 Multimode Detector (Beckman Coulter, Fullerton, California). STR amplification and fragment analysis were performed as described in [6].

Statistical analyses calculated were performed using Arlequin software v3.5 [7] included: allele frequency, observed heterozygosity and Hardy–Weinberg equilibrium. Forensic efficiency parameters included: power of discrimination for females and males and probability of exclusion of paternity [8] for duos and trios. Pairwise genetic distances (Fst) were calculated with the same software using available population databases for the same X-STR markers. Pairwise genetic distances were used to construct an unrooted neighbor-joining tree using TreeFit [9] and visualized on TreeView [10].

Allele frequency and forensic efficiency parameters (Supplementary Table 1) were obtained for a total of 344 X-chromosomes. Deviations from Hardy–Weinberg equilibrium were not observed at any locus. Acceptable levels of power of discrimination and mean exclusion chance were determined. The most polymorphic locus was DXS6789. The highest power of discrimination was

determined for loci DXS6809, GATA172D05 and GATA31E08, being GATA172D05 the most heterozygous. Combined power of discrimination was 99.9999997% and 99.9998548% for females and males, respectively. Mean exclusion chance was 99.9993172% (trios) and 99.9708265% (duos) (Supplementary Table 2).

Pairwise genetic distances (Fst) (Supplementary Table 3) indicate that allele frequencies are not significantly different ( $p \leq 0.005$ , after Bonferroni correction) from other Iberian populations (except Pas Valley, Navarre and Northern Portugal) and significantly different from all American populations. The unrooted neighbor-joining tree (Supplementary Figure 1) shows a close genetic distance to geographically distant populations with a non-isolated profile in the Iberian Peninsula, such as Barcelona, Cantabria, Galicia, and Central Portugal, and further from populations considered as genetic isolates, such as Navarre, the Basque Country and Pas Valley. In a previous report [6], western populations of the Iberian Peninsula were represented genetically closer to Brazilian populations (São Paulo, Mato Grosso and Rio de Janeiro) on an unrooted neighbor-joining. The Iberian populations were mostly represented by northern populations characterized by isolated genetic profiles, Pas Valley and Navarre being furthest from the rest. However, in this study, adding a population bearing a non-isolated profile like Murcia caused a rearrangement of the three western Iberian populations (Galicia, North and Central Portugal) closer to the rest of the Iberian populations showing a more accurate visualization of genetic distances in agreement with geographical regions. Results are consistent with previous reports [6,11,12] regarding genetic distances between Iberian and American populations based on the same X-STR panel and indicate a need to analyze other populations of the Iberian Peninsula with the same panel in order to better understand phylogenetic relations at a regional level and establish a reference database.

This paper follows the guidelines for publication of population data requested by the journal [13] and the International Society for Forensic Genetics (ISFG), as well as abiding to ISFG recommendations on the analysis of DNA polymorphisms [14].

**Competing interest**

Authors declare no competing interest in the content of this manuscript.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.fsigen.2012.06.010>

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