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Typing of Amerindian Kichwas and Mestizos from Ecuador with the SNP*for*ID multiplex

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ABSTRACT

A total of 119 unrelated individuals from two of the major ethnic groups in Ecuador were typed for 49 of the autosomal single nucleotide polymorphisms (SNPs) in the SNP*for*ID 52plex using the SNapShot® assay. Of the above, 42 samples originated from Mestizos (an admixed population) and the remaining 77 were from Native Amerindian Kichwas. We obtained full SNP profiles in all individuals and concordance of duplicated analyses. No deviation from Hardy–Weinberg equilibrium (HWE) was observed for any SNP in the Mestizo and Kichwa populations and only one and four pairs of loci, respectively showed significant linkage disequilibrium. A relatively low genetic diversity and global positive F_{IS} value was observed in Kichwas. A statistically significant global F_{ST} value was obtained when the two Ecuadorian populations were compared with populations in Spain, Portugal, Argentina, Denmark, Greenland, China, Somalia and Mozambique. All pairwise F_{ST} values were statistically significant. A multi-dimensional scaling based on pairwise F_{ST} values showed that the Kichwa population differed from all other populations investigated and that the Mestizos had an intermediate position between Kichwas and Europeans. An admixture analysis indicated that the greater contributor to the Mestizo population was the Kichwas (71.2%) compared to the European contribution. The combined mean match probability and mean paternity exclusion probability were 3.3×10^{-17} and 0.998, respectively, for the Mestizo population and 3.3×10^{-14} and 0.993, respectively, for the Kichwa population.

1. Population and samples

The population in Ecuador is a multicultural and pluriethnic population [1]. Currently, three main ethnical groups inhabit Ecuador [1–4]: (a) Mestizos, an admixed population group with descendents of Spanish Caucasian and Native Amerindians counting for around 60% of the population; (b) native Amerindians, comprised by 11 major groups and self-denominated, indigenous nationalities where the most numerous group is the Kichwa speaking group that represents around 20% of the Ecuadorian population; and (c) Afro-Ecuadorians who are descendants of African slaves. Studies performed on autosomal and Y-chromosome STRs showed a low genetic diversity in some of the Native Amerindians groups, a complex admixture and a high heterogeneity between the ethnic groups [2,3].

In order to study the genetic variability in Ecuadorian populations using the 49plex SNP assay [5], 119 blood samples (77 from Mestizos and 42 from Kichwas) were collected. Samples from Kichwas were obtained at their own communities in the western area of Ecuador (Kichwas from Oriente), while samples from Mestizos were collected in Quito. The ethnographic description criteria of the subjects in this study included skin colour, surname, town of origin, language and self-recognition of their identity. All the samples were obtained after informed, written consent, and all samples were donated by the owners for genetic analysis.

2. DNA extraction

DNA was extracted from four 1.2 mm disks punched from blood stains on FTA® cards (Whatman Inc., Clifton, NJ) using a Harris Micro-Punch® (Ted Pella Inc., Redding, CA). DNA extraction was performed using the DNA Investigator kit (Qiagen, GmbH, Hilden, Germany) and a BioRobot® EZ1 Workstation (Qiagen) as previously described [6]. A final elution volume of 50 μL autoclaved

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Milli-Q water was used. DNA concentration was estimated using the Quantifiler® Human DNA Quantification kit (AB: Applied Biosystems, Foster City, CA, USA) on an AB 7900 Real-Time PCR system (AB) according to the manufacturer's recommendations. DNA concentrations ranged from 4 pg/μL to 370 pg/μL.

2.1. SNP typing assay

Forty-nine of the 52 autosomal SNP*for*ID SNPs [7] were amplified in a single PCR as previously described [5]. PCRs were set up in duplicates. Two SBE reactions were performed for each PCR product using the SNaPshot® kit (AB) [5]. The SBE products were analysed by capillary electrophoresis with an ABI3130*xl* Genetic Analyzer (AB) with 36 cm capillary arrays and POP-4 polymer (AB). The electropherograms were analysed with GeneScan® 3.7 (AB) and Genotyper 3.7 software (AB). The criteria for acceptable peak height ratio for heterozygote allele calls and the acceptable signal/noise ratio for homozygous allele calls for each locus were as previously described [7]. Allele calls were made independently by two analysts and the results were only accepted if they were concordant.

2.2. Quality control

All samples were analysed at least twice and the results from the two experiments were compared. If the results differed, the sample was analysed up to four times. All experiments were performed at the Section of Forensic Genetics, Department of Forensic Medicine, University of Copenhagen that is accredited according to the ISO 17025 standard. The 49plex SNP assay was validated locally and through inter-laboratory, collaborative exercises prior to the typing of the two ethnical groups from Ecuador [5,7–10]. Detailed guidelines for the interpretation of the results of the SBE analysis were recently developed [5]. The recommendations of the International Society of Forensic Genetics on the analysis of DNA polymorphisms were followed including the use of recommended nomenclature and guidelines regarding quality control and statistical calculations [11].

2.3. Data and statistical analyses

The Arlequin 3.5 software [12] was used to perform most of the population analysis. Allele frequencies and expected and observed heterozygosities were calculated. Departure from Hardy–Weinberg equilibrium was tested as described by Guo and Thompsom [13]. Linkage disequilibrium between pairs of loci was tested using an extension of Fisher's exact test. Data regarding the 49 autosomal SNPs analysed in eight populations were retrieved from the SNP*for*ID browser [14]: (Spain, Portugal, Argentina and Mozambique) and from Sanchez et al. [7] (Denmark, Somalia, Greenland and China). A two-hierarchical AMOVA analysis was performed in order to estimate the population genetic structure. Fixation indices F_{IS} and F_{ST} and pairwise F_{ST} values were calculated and their significance was tested using 10,000 permutations. A multi-dimensional scaling (MDS) was drawn from the pairwise distances using the SPSS statistical package (SPSS Inc., Chicago, IL, USA). Genetic admixture was studied in the Mestizo population by using the Admix 2.0 software [15]. In this case the molecular distances between alleles were not taken into account. Two parental populations (Kichwa and Spanish) were considered for the admixture analysis. The step-down Sidak–Holm procedure was used to adjust for multiple tests [16,17]. The Combined Matching Probability (CMP), Power of Exclusion (PE) and Paternity Index (PI) were calculated using DNAVIEW 28.103 [18].

3. Results and discussion

SNP-types and allele frequencies for the 49 SNPs in the Kichwa and Mestizo populations are provided in Supplementary materials 1 and 2. The data is freely available online at the SNP*for*ID frequency browser [14].

No significant deviations from HWE were observed in Kichwas and Mestizos after correction for multiple testing. A slightly significant ($P < 0.05$), positive F_{IS} value was observed in the Kichwa population, which indicates an overall deficit of heterozygotes in this population (Supplementary material 2). Although the Kichwa population is estimated to account for nearly 92% of the Native Amerindians in Ecuador [1], they live in isolated populations and a certain degree of inbreeding is expected in each of the isolated populations. Nevertheless, the fact that around 37.5% of the 49 SNP loci showed F_{IS} values equal or less than 0 indicates that the overall deficit of heterozygotes could be due to subpopulation structure (Wahlund effect) as it was suggested for other Amerindian populations [19]. The average gene diversity index observed for the 49 loci was 0.304 and 0.389 in the Kichwa and Mestizo populations, respectively. These results are in agreement with previous studies [2,3] where the Kichwa population showed a decreased genetic diversity, as reported for other Amerindian populations. One of the 49 SNP loci was found to be monomorphic in Kichwas (rs2056277) and 15 out of 49 SNP loci showed minimum allele frequencies lower than 10% (Supplementary material 2). Locus rs2056277 showed the lowest minimum allele frequency in Mestizos (8.3%), and five of the 49 SNP loci showed minimum allele frequencies under 10% in the Mestizo population.

Significant linkage disequilibrium ($P < 0.01$) was found after correction for multiple comparisons in four of the 1176 pairwise comparisons in the Kichwa population (rs826472–rs251934, rs2830795–rs964681, rs717302–rs907100 and rs251934–rs914165). After correction, one pair of loci (rs717302–rs763869) showed significant linkage disequilibrium ($P < 0.01$) in Mestizos. These SNPs are positioned on chromosomes 5 and 8, respectively.

The amount of genetic structure was estimated for 10 populations (Kichwas and Mestizos from Ecuador, Argentina, Spain, Portugal, Denmark, Greenland, China, Mozambique and Somalia). A significant overall F_{ST} was observed ($F_{ST} = 0.09$, $P < 0.00001$). Significant pairwise F_{ST} values were obtained after correction for all population pairs ($P < 0.01$) (Supplementary material 3). A multi-dimensional scaling (MDS) was drawn from the pairwise F_{ST} values (Supplementary material 4). The 10 populations included in the analyses showed a spread distribution with a general low tendency to group. Short distances were observed between the three European populations and between Mestizos and Argentineans. The greatest F_{ST} value was observed between the Mozambique population and the Kichwas. Regarding the two Ecuadorian groups, the relative position of the Kichwas and Mestizos was in agreement with previous studies [2,3]. While the Kichwas showed a clear displacement from the other nine populations, the Mestizo population showed an intermediate position between Kichwas and Europeans, with a greater F_{ST} value between Mestizos and Spaniards than between Mestizos and Kichwas.

Admixture proportions in the Mestizo population were estimated by using Kichwas and Spaniards as parental populations. As calculated for autosomal STRs, the greater contributor to the Mestizo population turned out to be the Kichwas (71.2%; SD = 3.1%). The contribution of the Spanish population was estimated to be 28.8% (SD = 3.1%). Previous studies estimated a West African contribution to the Mestizo population of around 8% [3]. Due to the limited amount of data available of the 49 SNPs in West African populations, we did not include this third possible population source in the admixture analysis.

It is interesting to note that the pairwise F_{ST} values were 3–4 times higher for Kichwas, Mestizos and Spaniards when they were calculated from 49 autosomal SNPs data than when the values were calculated from 15 autosomal STRs [3]. The difference could be explained by the nature of the two sets of markers (i.e., a much higher mutation rate and higher intrapopulation variability observed for STRs than for SNPs).

Using the 49 SNPs, the combined mean match probability and mean paternity exclusion probability were 3.3×10^{-17} and 0.998, respectively, for the Mestizo population and 3.3×10^{-14} and 0.993, respectively, for the Kichwa population. The typical paternity indices, i.e., the geometric mean, for trios and duos (motherless) were 124,000 and 1500, respectively, for Mestizos and 23,300 and 541, respectively for Kichwas.

In summary, a complex scenario was observed in two Ecuadorian sub-populations, which points out the need of local databases for correct application in forensic practice. Even given the relatively low genetic diversity observed especially in Kichwas, the forensic usefulness of the 49 SNPs is still remarkable in the Ecuadorian populations.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.fsigen.2011.03.006.

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