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Short communication

Analysis of 49 autosomal SNPs in an Iraqi population

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ABSTRACT

Forty-nine of the 52 autosomal single nucleotide polymorphisms (SNPs) in the SNP*for*ID 52plex were typed in 101 unrelated Iraqis living in Denmark. No significant deviation from HWE was found in all but one of the 49 SNP systems and no significant pairwise linkage disequilibrium was observed for any SNP pair. When 18 worldwide populations were compared (including populations in Iraq, Turkey, Israel, Pakistan, India, China, Taiwan, Japan, Siberia, Algeria, Somalia, Uganda, Mozambique, Angola, Nigeria, Denmark, Portugal, Spain), a significant global F_{ST} value was obtained. All but six F_{ST} values were statistically significant when pairwise comparisons were performed between the 18 populations. The Iraqi population did not show significant difference from the population in Turkey and it grouped together with other Middle-Eastern populations when a multidimensional scaling plot was drawn based on the pairwise F_{ST} values. The combined mean match probability and the typical paternity index for trios were 8.3×10^{-20} and 259,000, respectively, for the Iraqi population.

1. Population and samples

The Iraqi population is heterogeneous and includes a number of ethnic groups. Around 75–80% of the total Iraqi population are Arabs. Kurds represent 15–20% of the total population, while approximately 5% of the population is constituted by minority groups such as Turkomans, Yazidis, Assyrians and Armenians [1]. According to the Danish Statistic Bank ("Statistikbanken®" [2]), 29,859 individuals from Iraq were registered as residents in Denmark in July 2011. In order to establish a frequency database for 49 autosomal SNPs [3] for the Iraqi community residing in Denmark, blood samples from 101 presumably unrelated Iraqis involved in immigration cases were collected. The work was approved by the Danish ethical committee (H-1-2011-081).

2. DNA extraction

DNA from approximately half the samples was extracted from 200 μL blood using the QIAamp DNA blood mini kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's recommendations. DNA from the other half of the samples was extracted from 4 μL of blood using the DNA Investigator kit (Qiagen) and a BioRobot® EZ1 Workstation (Qiagen) according to the manufacturer's instructions.

3. SNP genotyping and quality control

Forty-nine of the 52 autosomal SNP*for*ID SNPs [4] were analyzed using a single PCR and two single base extension (SBE) reactions as previously described [3]. The SBE products were run in an ABI3130*xl* Genetic Analyzer (AB) with 36 cm capillary arrays and POP-4™ polymer (AB). The GeneScan® 3.7 (AB) and Genotyper® 3.7 software (AB) were used to analyze the resulting electropherograms. Previously described criteria for homo- and heterozygote allele calling were used [4]. Each sample was run two times. Allele calls were independently made by two analysts and the results were compared. All experiments were performed in the laboratory of the Section of Forensic Genetics, Department of Forensic Medicine, Faculty of Health Sciences, University of Copenhagen, which is accredited according to the ISO 17025 standard.

4. Data analyses

Population genetic analyses were performed using Arlequin 3.5.1.2 software [5]. Possible departures from Hardy–Weinberg equilibrium (HWE) were tested as described by Guo and Thompsom [6]. Linkage disequilibrium between pairs of loci was tested using an extension of the Fisher's exact test [7]. Data regarding the 49 autosomal SNPs analyzed in populations from Turkey, Israel, Pakistan, India, China, Taiwan, Japan, Siberia, Algeria, Somalia, Uganda, Mozambique, Angola, Nigeria, Denmark, Portugal and Spain were collected from the SNP*for*ID browser [8]. Samples with more than two missing results were removed from the analyses. The global amount of population genetic structure

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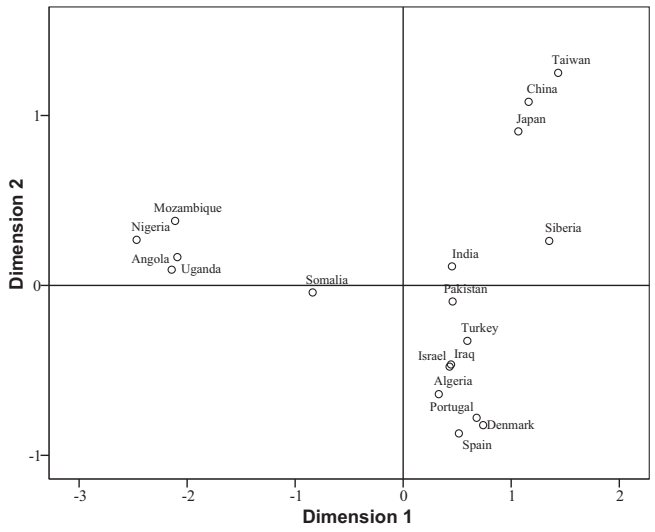

Fig. 1. Multidimensional scaling plot based on pairwise F_{ST} values calculated on the Iraqi and 17 other populations typed for 49 autosomal SNPs.

was estimated using a two-hierarchical AMOVA analysis. Pairwise F_{ST} values were calculated and their significance was tested using 10,000 permutations. A multidimensional scaling (MDS) was drawn from the pairwise F_{ST} values using the SPSS statistical package (SPSS Inc., Chicago, IL, USA). The step-down Holm-Sidak procedure was used to adjustment for multiple tests [9]. Forensic parameters of interest, such as the Combined Matching Probability (CMP), Power of Exclusion (PE) and Paternity index (PI), were calculated using DNAVIEW 28.103 [10].

5. Results and discussion

Allele frequencies and observed and expected heterozygosities for the 49 SNPs studied in the Iraqi population are provided in Supplementary Table 1. The minimum allele frequency observed for the 49 autosomal SNPs analyzed in the Iraqi population varied from 0.178 (rs938283) to 0.500 (rs8037429). All but one SNP (rs2107612) showed the number of heterozygotes expected under HWE. The heterozygosity observed for the SNP system rs2107612 (0.287) was significantly lower ($P < 0.05$, after step-down Holm-Sidak correction) than expected (0.431). Technical problems such as possible null alleles do not seem to be the explanation for the deficit of heterozygotes observed for this system. A significant low number of heterozygotes were not observed for the rs2107612 system in any of the 17 populations collected from the SNP*for*ID browser [8]. It is possible that, as a result of the ethnical heterogeneity of the Iraqi population, an excess of homozygotes is observed for the SNP marker rs2107612 when the global population is analyzed (Whalund effect). A positive, non significant F_{IS} value was obtained for the 49 SNPs in the Iraqi population ($F_{IS} = 0.038$). No significant linkage disequilibrium was found after correction in 1176 pairwise comparisons.

A two-hierarchical AMOVA analysis was performed in 18 populations from Iraq, Turkey, Israel, Pakistan, India, China, Taiwan, Japan, Siberia, Algeria, Somalia, Uganda, Mozambique, Angola, Nigeria, Denmark, Portugal and Spain. A significant overall F_{ST} value was obtained ($F_{ST} = 0.067$, $P < 0.00001$). From the 153 pairwise F_{ST} values (Supplementary Table 2), all but 6 comparisons were statistically significant ($P < 0.05$, after step-down Holm-Sidak correction). In contrast to the results obtained in previous studies performed with autosomal STRs [11], no significant difference was observed between the Iraqi and Turkish populations. The pairwise F_{ST} values were represented in an MDS plot (Fig. 1). The 18 populations included in the analysis were mainly placed according to their geographical origin. The Iraqi population grouped closely together to other Middle-Eastern populations included in the study.

The combined mean match probability using the 49 SNPs was 8.3×10^{-20} corresponding to a combined power of discrimination of >99.99999%. The typical paternity indices for trios and duos obtained with the 49 SNPs were 259,000 and 2880, respectively. The mean exclusion probability was 99.96%.

In summary, we present a frequency database for 49 autosomal SNPs for the Iraqi community residing in Denmark. Even though the Iraqi population sample analyzed in this study was close to other Middle-East populations, our database may not well represent the Iraqi population. The ethnical complexity described for the population in Iraq may require the development of frequency databases that takes the heterogeneity of this population into account.

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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.2012.05.004.

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