Genetic ancestry of a Moroccan population as inferred from autosomal STRs

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Abstract

Detecting population substructure and ancestry is a critical issue for both association studies of health behaviors and forensic genetics. Determining aspects of a population’s genetic history as potential sources of substructure can aid in design of future genetic studies. Within this context, fifteen autosomal short tandem repeat (STR), were used to examine population genetic structure and hypotheses of the origin of the modern Moroccan population from individuals belonging to three different ethnical groups from Morocco (Arab, Berber and Sahrawi), by comparing their autosomal STR variation with that of neighboring and non-neighboring populations in North Africa, Europe and Middle East as well as proposed ancestral populations in Morocco (Berber). We report on the results that the gradient of North African ancestry accounts for previous observations of low levels of sharing with Near East and a substantially increased gene flow especially from Morocco and Spain.

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Introduction

The question of Moroccan ancestry has been addressed for ages to answer questions of human evolutionary, historical and medical significance. Human evolutionary or anthropological studies have focused on vast array of DNA-based genetic markers, each of which has different attributes but complementary roles (Garrigan and Hammer, 2006). The nuclear genome contains the higher rate of polymorphisms. These include microsatellites that consist of tandemly repeated DNA sequences with a
variable number of repeats from one individual to another. Thousands of microsatellite polymorphisms have now been identified in human. Their mutation rate is much higher than that of single nucleotides, approaching $10^{-3}$ per generation (White et al., 2007; Zhang and Hewitt, 2003). Because of this high mutation rate, microsatellites have the potential to provide information about recent evolutionary events which made them the markers of choice in population genetics and forensic applications (El Amri et al., 2011, Cibbel et al., 2003). By comparing the autosomal and X chromosome, it has been clear that the lower mutation rate, smaller population size and higher linkage disequilibrium make the X chromosome less informative than autosomes but record substantially older histories than the Y chromosome (Bentayebi et al., 2012a, 2012b). In the other hand, the mitochondrial genome as well as the Y chromosome offer a very different perspective on human evolution. The absence of recombination in these regions of the genome and their uniparental characteristics (maternal for the mtDNA and paternal for the Y-chromosomal DNA) allows researchers to infer past human behaviors and evolutionary events such as migrations, founder events, population bottlenecks or expansions, relative male and female contributions to an admixed population, marriage practices, and mode of transmission of languages (Underhill and Kivisild, 2007; Xue et al., 2008). Interestingly, a joint analysis has the potential of providing more robust information (Jorde et al., 1998), as these markers give an independent (for autosomal and X chromosome) and dependent (for mitochondrial DNA and Y chromosome) picture of sex-specific demography (Ségurel et al., 2008; Ouborg et al., 2008, White et al., 1998, Schaffner, 2004; Bentayebi et al., 2012a, 2012b). Mitochondrial DNA studies of Moroccan population, at times, provided interesting results that together with autosomal and other genetic markers will help to understand the genetic landscape of the Moroccan population. In molecular evolution, a haplogroup is a group of similar haplotypes that share a common ancestor having the same polymorphism in all haplotypes. Some haplogroups exhibit specific geographic homelands (Aboukhalid et al., 2013). Haplogroup U6 in the mtDNA phylogenetic tree descends from the haplogroup U, who lived around 55,000 years ago. Recent studies based on the distribution of mtDNA of haplogroup U6 raised two theories about the origins of modern human populations in North Africa. The first put forward that groups of the proto-U6 lineage spread from the Near East to North Africa around 40–45 ka (thousands of years ago) (Olivieri et al., 2006), followed by some degree of regional continuity, while the second proposes a westward human migration from the Near East, followed by further demographic expansion at 22 ka centered on the Maghreb and associated with a microlithic bladelet culture known as the Iberomaurusian (Maca-Meyer et al., 2003; Pereira et al., 2003). In the other hand, a recent leading evolutionary theory envisages that the first modern humans in Europe, called the Cro-Magnon (Iberomaurusian), arrived from North-west Africa and are believed to have completely replaced the previous inhabitants “the Neanderthals” (Caramelli et al., 2008). Present day genetic diversity of North African populations has been revised through a wide variety of uniparental and autosomal genetic markers.

Morocco is an appropriate region for population genetic studies and a good example for answering and completing the previous questions and theories. Located in northwest Africa with a total of 13 living languages listed by Ethnologue (Gordon, 2005), Morocco is important for studies concerning human migration, because it is bounded by the Mediterranean Sea to the North with just 14 km away from Spain across the Gibraltar strait and the Atlantic Ocean to the west, which is part of the traditionally favored model of the migratory route out of Africa for anatomically modern humans (Bilogili and Weyel, 2009). Berber-speaking populations, as representative of the autochthonous inhabitants of North Africa, inhabit a wide ranging area extended from the Saharan desert to the Atlas Mountains and nowadays combining six different countries from Egypt to Mauritania, speaking 26 sorts of Berber dialects coming all from a unique and standard Berber language (Gordon, 2005; Hayward, 2000; Louali and Philippson, 2003). In spite of their self-identification by their common language, Berbers have a complex history of invasions, conquests and migrations, especially the Arabic conquest in the seventh century AD. The Arab-Muslim conquerors adopted a language policy that enabled them to spread Arabic and Islamic cultural values (Brunschiwig R, 1975). However, today in Morocco, beside Standard Arabic, Berber language remains one of the two national languages of Morocco (Gordon, 2005).

The considerable ethnic and cultural diversity within Morocco reflects differential historic population influences and make the study of existing genetic diversity of the kingdom an attractive effort. Morocco has the most complex linguistic situation compared to the three other North African countries with a remarkably unequal repartition of population: 90% of residents settle in the north part of Morocco. Although around half of the total population speaks one of the different Berber dialects, it is quite difficult
to trace a clear line among Berber, Arabized Berber and Arabic people in Morocco (Abderrahman Z, 2013). All these historical events together with recent demographical movements have left an important imprinting in their current genetic background.

Previous genetic studies in Morocco have mainly focused on classical markers, Alu polymorphisms (Aboukhalid et al., 2013; Bentayebi et al., 2011), mitochondrial DNA (Bentayebi et al., 2012a, 2012b), X chromosome STRs and SNPs (Picornell et al., 2011; Tomas et al., 2008) and Y-chromosome STRs and SNP (Aboukhalid et al., 2009, 2010) variation in Berber and Arabic speaking samples from small and limited parts of Morocco. The present work completes the previous studies investigating a wide range of samples from different part of Morocco (Arab, Berber and Sahrawi).

Analyses of autosomal STRs have become very useful tools, both in evolutionary studies and forensic casework (Gaibar et al., 2010). In the same way, AmpFISTR Identifiler PCR amplification kit provides an easily reproducible and highly reliable method for typing 15 highly polymorphic STR loci for forensic and evolutionary purposes (Anslinger et al., 2001; Applied Biosystems, 2001). The aim of this study is to analyze STR genotypes in a Moroccan population that has special interest because of its particular geographical, cultural and historical characteristics (Fernández-Santander et al., 1999).

The present study characterizes autosomal STR genotypes from Morocco to examine population substructure and genetic relationships with other groups, including testing of the proposed genetic affinities between Morocco and populations in Southern Europe, North Africa and Middle East (Aboukhalid et al., 2009, 2010, 2013; Bentayebi et al., 2011, 2012a, 2012b; Picornell et al., 2011). We predict that if the actual Moroccan population share a common ancestor (or have experienced more recent migration and resultant gene flow) with either populations in Southern Europe or Middle East (Arab), allele frequencies of autosomal STR loci will be similar among Morocco and these proposed related populations and genetic distances between these populations will be low. Alternatively, if Morocco is an autochthonous North African population (with no recent gene flow from groups in Middle East or Europe), autosomal STR frequencies will be within the range of other populations in North Africa, and Moroccan will be more genetically similar to other North African groups with a Berber ancestry. Previous studies presenting STR data from the Moroccan population either used small samples that were often collected in urban areas of a single province to study relationships between Morocco and other populations (Chbel et al., 2003; El Ossmani et al., 2009, 2010) (Table 1). This study represents one of the most comprehensive samples of Morocco yet analyzed for autosomal STR variation, with 377 male and female throughout the country.

Materiel and methods

DNA samples

Blood samples and buccal swabs were collected from 320 healthy unrelated men and women (men n = 170, women n = 150) belonging to the three ethnic groups (Arab n = 116, Berber n = 104, Sahrawi n = 100) living in Morocco and including 145 samples already typed for 12 X-STRs (Bentayebi et al., 2012a, 2012b), were analyzed. Genomic DNA was extracted using a DNA IQ™ System (Promega, Madison, USA) according to the manufacturer’s instructions, and the DNA was quantitated using a NanoDrop 8000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA), following the manufacturer’s

| Size | Ethnicity               | Location                                      |
|------|-------------------------|-----------------------------------------------|
| 138  | Unknown ethnicity       | Moroccan living in Brussel Abdin et al. (2003) |
| 180  | Arab, Berber and Saharawi | Various parts of Morocco El Amri et al. (2011) |
| 201  | Berber                  | Azrou (Atlas Montains) El Ossmani et al. (2010) |
| 204  | Arab                    | Southern Morocco El Ossmani et al. (2009)     |
| 234  | Arab                    | Casablanca (Central Morocco) Chbel et al. (2003) |
| 387  | Arab                    | Rabat, Sale, Zemmour, Zaer (Central Morocco) El Ossmani et al. (2008) |
recommendation. Samples were obtained, with informed consent. All participants had all four of their grandparents born in the same region. The work was approved by the Ethical Committee of the University of Mohammed V-Agdal. Information about the linguistic origins was also recorded.

**Polymerase chain reaction (PCR) amplification and genotyping**

PCR amplification was performed according to the manufacturer’s instruction (AmpFISTR Identifiler™, Applied Biosystems, Foster City, CA, USA), to amplify the 15 autosomal short tandem repeats (CSF1PO, D2S1338, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D19S433, D21S11, FGA, TH01, TPOX, vWA) included in the AmpFISTR Identifiler PCR Amplification Kit™. The samples were amplified in single reactions for all loci using fluorescently labeled primers. Electrophoresis was performed on an ABI Prism 3130 XL Genetic Analyser (Applied Biosystems, Foster City, CA).

**Comparative population data**

To test hypotheses of population origins, autosomal STR data from our sample were compared to geographically assumed-related populations. In this way, data on 13 STRs (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, VWA, TPOX, D18S51, FGA, D5S818, D16S539) in a total of 21 populations, were selected from the available literature and compared to our sample, including populations from North Africa (Moroccan Berber, Tunisian Berber, Tunisian Arabic and Libyan) (Cherni et al., 2005; El Ossmani et al., 2010; Khodjet-el-Kkil et al., 2012); Southern Europe (Italy, Spain and Greece) (Brisighelli et al., 2009; Camacho et al., 2007; Kovatsi et al., 2006); and Middle East (Palestine, Syria, Lebanon, UAE, Saudi Arabia, Oman, Yemen, Iraq, Kuwait, Egypt and Sudan) (Abdin et al., 2003; Abu Halima et al., 2009; Alenizi et al., 2008; Alshamali et al., 2005; Babiker et al., 2011; Barni et al., 2007; Chouery et al., 2010; Omran et al., 2009) and Western Asia (Pakistan, Iran, Fars) (Alshamali et al., 2005; Hedjazi et al., 2013; Rakha et al., 2009).

**Statistical analysis**

Arlequin software ver 3.11 (Excoffier et al., 2005) was used to calculate allele frequencies, population pairwise genetic distances (FST), AMOVA test, and also to assess departures from Hardy–Weinberg equilibrium. Pairwise FST values for each STR were calculated between 22 populations, based on 13 of the 15 studied STRs (D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D13S317, D7S820, TH01, TPOX, CSF1PO, D19S433, D2S1338). They were averaged over loci and represented in a multidimensional scaling (MDS) plot using the statistical package SPSS v.17.0 (Levesque, 2007).

Statistical parameters of forensic interest (PI, paternity index; MP, matching probability; PD, power of discrimination; PIC, polymorphic information content and PE, power of exclusion) were calculated using PowerStats v1.2 (Promega Corporation, Madison, WI) software package (Tereba, 1999). They are defined as follows (Houston, 1998):

Matching probability (MP), also known as probability of match (pM), is the number of individuals that may be surveyed before finding the same DNA pattern in a randomly selected individual. This is represented as:

\[
MP = \sum_{i=1}^{n} \sum_{j=1}^{n} P_{ij}^2
\]

where i and j represent the frequencies of all possible alleles a through n, Pij represents the frequencies of all possible genotypes.

The paternity index (PI) reflects how many more times likely it is that the person being tested is the biological father, rather than a randomly selected individual. The typical paternity index is assigned to a locus rather than an individual case. Generally, a PI of less than one is indicative of non-relatedness. The PI is represented by the following equation:

\[
PI = \frac{1}{2H}
\]

where H is the frequency of homozygotes.
The power of discrimination is defined as the probability of discrimination between two unrelated individuals. Thus, correlated to MP discussed above:

\[ PD = 1 - MP. \]

The power of exclusion (PE) is defined as the fraction of individuals having a DNA profile that is different from that of a randomly selected individual in a typical paternity case. The value for each individual case will vary. The average for a given locus is represented by the following equation:

\[ PE = h^2 \left( 1 - 2hH^2 \right) \]

where \( h \) is the heterozygosity and \( H \) is the frequency of homozygotes.

The polymorphism information content (PIC) is the probability that one could identify which homologue of a given parent was transmitted to a given offspring, the other parent being genotyped as well.

\[ PIC = 1 - \sum_{i=1}^{n} p_i^2 - \sum_{i=1}^{n} \sum_{j=i+1}^{n} 2p_i^2p_j^2. \]

**Results**

Allele frequencies of 15 autosomal STR loci (D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820, TH01, TPOX, CSF1PO, D19S433, D21S1338, D16S539) as well as the values for forensic parameters are shown in Table 2. D21S11 was the most polymorphic autosomal STR, with 18 alleles, while TH01, TPOX, CSF1PO, D5S818, D7S820 and D16S539 were the least polymorphic with 6 alleles each. In particular, the most frequently observed alleles of the TH01, TPOX, CSF1PO, D5S818, D7S820 and D16S539 loci had 23.0%, 41.0%, 34.4%, 39.2%, 34.0% and 30.5% occurrences, respectively. Informativeness can be quantitatively measured by the polymorphism information content or PIC. Theoretically, PIC values can range from 0 to 1. At a PIC of 0, the marker has only one allele. At a PIC of 1, the marker would have an infinite number of alleles. A PIC value of greater than 0.7 is considered to be highly informative. Clearly markers with greater numbers of alleles tend to have higher PIC values and thus are more informative (Hildebrand et al., 1992). Using the equation for PIC, eleven autosomal markers included in the Identifiler are considered to be highly polymorphic: 73% of the offspring should be informative for D3S1358, 79% for vWA, 82.2% for FGA, 81.9% for TH01, 72.5% for D7S820, 80% for D8S1179, 80.1% for D21S11, 85.5% for D18S51, 72.7% for D16S539, 81.8% for D21S1338 and 80.1% for D19S433. While only four markers are considered to be moderately polymorphic: 68% of the offspring should be informative for TPOX, 66% for CSF1PO, 69.9% for D5S818 and 68.9% for D13S317. No significant deviations from the Hardy–Weinberg equilibrium were observed in these 15 markers (Table 2).

The observed heterozygosity (Ho) ranges from 0.737 (CSF1PO) to 0.860 (FGA). The power of discrimination varies between 0.855 (CSF1PO) and 0.970 (D18S51), and the combined power of discrimination for the 15 STR loci is 0.99999999. The probability of excluding paternity (PE) varies between 0.400 (TPOX) and 0.675 (TH01), and the combined probability of excluding paternity for the 15 loci is 0.999. The present work of the Moroccan population was conducted in order to complete the STR profiling of the different ethnic groups of the country. Previous studies were published on the Arab and Berber speaking groups (Chbel et al., 2003; El Ossmani et al., 2010) but this is the first large work that encloses both groups plus a small Sahraoui pool. All statistical values reported in this work showed no significant difference from those reported precedently (Table 2).

A first approach to population diversity by locus through the Fst statistic values range from −0.56% for vWA between Morocco and Libya to an impressive 11.8% for TPOX between Morocco and Pakistan. A hierarchical AMOVA, assuming four geographical groups (‘Maghreb’, “South Europe”, “Middle East” and ‘Western Asia’), does not reveal any significant distances between the Mediterranean populations. The frequency variance between the four groups (Fct = 0.3%, P = 0.154) being clearly lower than the diversity among populations within groups (Fsc = 0.44%, P = 0.068) (Table 3).
### Table 2
Allele frequencies and statistical parameters of AmpFlSTR Identifier PCR Amplification Kit loci in Moroccan population.

| Locus     | D3S1358 | vWA  | FGA   | TH01  | TPOX  | CSF1PO | D5S818 | D13S317 | D7S820 | D8S1179 | D21S11 | D18S51 | D16S539 | D2S1338 | D19S433 |
|-----------|---------|------|-------|-------|-------|--------|--------|---------|--------|---------|--------|--------|---------|---------|---------|
| Genotype  | 0.006   | 0.020 | 0.006 | 0.006 | 0.001 | 0.006  | 0.008  | 0.005   | 0.013  | 0.006   | 0.002  | 0.006  | 0.001   | 0.003   | 0.003   |
| Genotype  | 0.002   | 0.230 | 0.027 | 0.017 | 0.002 | 0.005  | 0.013  | 0.006   | 0.013  | 0.006   | 0.002  | 0.006  | 0.001   | 0.003   | 0.003   |
| Genotype  | 0.018   | 0.195 | 0.410 | 0.027 | 0.050 | 0.074  | 0.154  | 0.001   | 0.002  | 0.154   | 0.001  | 0.006  | 0.017   | 0.002   | 0.005   |
| Genotype  | 0.003   | 0.252 | 0.193 | 0.025 | 0.035 | 0.051  | 0.089  | 0.001   | 0.002  | 0.089   | 0.001  | 0.006  | 0.017   | 0.002   | 0.005   |
| Genotype  | 0.003   | 0.134 | 0.080 | 0.271 | 0.306 | 0.065  | 0.047  | 0.340   | 0.100  | 0.001   | 0.005  | 0.054  | 0.014   | 0.013   | 0.014   |
| Genotype  | 0.280   | 0.197 | 0.001 | 0.147 | 0.003 | 0.150  | 0.004  | 0.010   | 0.140  | 0.004   | 0.027  | 0.070  | 0.002   | 0.004   | 0.004   |
| Genotype  | 0.261   | 0.226 | 0.197 | 0.001 | 0.147 | 0.003 | 0.150 | 0.004   | 0.027  | 0.070   | 0.002  | 0.004  | 0.004   | 0.004   | 0.004   |
| Genotype  | 0.243   | 0.232 | 0.003 | 0.003 | 0.003 | 0.003 | 0.003 | 0.003   | 0.003  | 0.003   | 0.002  | 0.004  | 0.004   | 0.004   | 0.004   |
| Genotype  | 0.142   | 0.148 | 0.003 | 0.003 | 0.003 | 0.003 | 0.003 | 0.003   | 0.003  | 0.003   | 0.002  | 0.004  | 0.004   | 0.004   | 0.004   |
| Genotype  | 0.020   | 0.044 | 0.003 | 0.036 | 0.017 | 0.002 | 0.002 | 0.002   | 0.002  | 0.002   | 0.002  | 0.002  | 0.002   | 0.002   | 0.002   |
| Genotype  | 0.159   | 0.001 | 0.001 | 0.138 | 0.002 | 0.060 | 0.090 | 0.001   | 0.001  | 0.090   | 0.001  | 0.001  | 0.001   | 0.001   | 0.001   |
| Genotype  | 0.210   | 0.001 | 0.001 | 0.138 | 0.002 | 0.060 | 0.090 | 0.001   | 0.001  | 0.090   | 0.001  | 0.001  | 0.001   | 0.001   | 0.001   |
| Genotype  | 0.201   | 0.001 | 0.001 | 0.138 | 0.002 | 0.060 | 0.090 | 0.001   | 0.001  | 0.090   | 0.001  | 0.001  | 0.001   | 0.001   | 0.001   |
Ho, observed heterozygosity; He, expected heterozygosity; PI, paternity index; MP, matching probability; PD, power of discrimination; PIC, polymorphic information content and PE, power of exclusion.
The allele frequencies of the 15 autosomal STR loci were compared with the exact test of population differentiation. We found no significant differences between Moroccan and Spanish populations in 10 loci ($P < 0.05$); $P$-values of population comparison were 0.09910 (D3S1358), 0.15315 (vWA), 0.62162 (FGA), 0.09910 (TH01), 0.00000 (TPOX), 0.37838 (CSF1PO), 0.38739 (D5S818), 0.00901 (D13S317), 0.00000 (D7S820), 0.29730 (D8S1179), 0.90090 (D21S11), 0.88288 (D18S51) and 0.62162 (D16S539). While the

Table 3
Locus-by-locus analysis of molecular variance of 13 autosomal short tandem repeat loci.

| Locus | Among groups | | | | Among populations | | | | Within populations | |
|-------|--------------|---|---|---|-------------------|---|---|---|-------------------|---|
|       | Percent variance | $F_{CT}$ | $P$ | Percent variance | $F_{SC}$ | $P$ | Percent variance | $F_{ST}$ | $P$ |
| 1     | 0.29          | 0.0029  | 0.0010 | 0.0000 | 0.0001 | 0.4448 | 99.71 | 0.0029 | 0.0176 |
| 2     | 0.27          | 0.0027  | 0.0000 | 0.0008 | 0.19844 | 99.65 | 0.00348 | 0.00000 |
| 3     | 0.05          | 0.0005  | 0.52688 | 1.57 | 0.01574 | 0.00000 | 98.38 | 0.01619 | 0.00000 |
| 4     | 0.71          | 0.0071  | 0.04301 | 1.02 | 0.01032 | 0.00000 | 98.27 | 0.01732 | 0.00000 |
| 5     | 1.03          | 0.0103  | 0.04692 | 1.38 | 0.01397 | 0.00000 | 97.59 | 0.02415 | 0.00000 |
| 6     | 0.17          | 0.0017  | 0.03617 | 99.54 | 0.00168 | 0.01564 | 0.17 | 0.00457 | 0.00000 |
| 7     | 0.07          | 0.0007  | 0.20821 | 0.35 | 0.00346 | 0.00098 | 99.58 | 0.00415 | 0.00000 |
| 8     | 0.13          | 0.0013  | 0.08309 | 0.22 | 0.00216 | 0.02444 | 99.65 | 0.00348 | 0.00000 |
| 9     | 0.31          | 0.0031  | 0.00098 | 0.17 | 0.00175 | 0.04790 | 99.51 | 0.00485 | 0.00000 |
| 10    | 0.53          | 0.0053  | 0.00098 | 0.16 | 0.00165 | 0.02053 | 99.31 | 0.00691 | 0.00000 |
| 11    | 0.03          | 0.0003  | 0.45846 | 0.14 | 0.00144 | 0.03617 | 99.82 | 0.00177 | 0.01564 |
| 12    | 0.02          | 0.0002  | 0.59922 | 0.30 | 0.00298 | 0.00000 | 99.73 | 0.00274 | 0.00000 |
| 13    | 0.37          | 0.0037  | 0.00098 | 0.11 | 0.00111 | 0.09580 | 99.52 | 0.00482 | 0.00000 |
| Global estimates | 0.0030 | 0.1540 | 0.0044 | 0.0680 | 0.0075 | 0.0030 |
| Covariance estimates | $V_a = 0.0012$ | $V_b = 0.0018$ | $V_c = 0.3944$ |

*Abbreviations: $F_{CT}$ — fixation index among groups; $F_{SC}$ — fixation index among populations within groups; and $F_{ST}$ — fixation index within populations.

The allele frequencies of the 15 autosomal STR loci were compared with the exact test of population differentiation. We found no significant differences between Moroccan and Spanish populations in 10 loci ($P < 0.05$); $P$-values of population comparison were 0.09910 (D3S1358), 0.15315 (vWA), 0.62162 (FGA), 0.09910 (TH01), 0.00000 (TPOX), 0.37838 (CSF1PO), 0.38739 (D5S818), 0.00901 (D13S317), 0.00000 (D7S820), 0.29730 (D8S1179), 0.90090 (D21S11), 0.88288 (D18S51) and 0.62162 (D16S539). While the
Iraqi population showed no significant differences with both Moroccan and Spanish populations in 9 loci. However, the Pakistani population showed significant differences from the Moroccan population \((P < 0.05)\) in most loci. The MDS analysis showed that the Moroccan population form a separated plot with the Iberian Peninsula, while populations from Near East and south east Europe were clustered together. Tunisian Arabic remains isolated at the opposite side of the MDS (Fig. 1).

**Discussion**

Our study of the autosomal STR variation supports the hypotheses of a recent common ancestor between North Africa and Southern Europe (Morjani et al., 2011). The deviation of Arabic Tunisian was of particular concern.

This paper describes the pattern of the frequency distribution of 15 polymorphic STR of the autosomal chromosomes in the Moroccan population. In general, the allele frequencies found range within the general patterns described previously, but with a remarkable between population variations. In terms of variation, the samples were adjusted to a decreasing pattern of diversity from East to West (mean heterozygosity for Kuwait: 0.875, Syria: 0.822, Italy: 0.796 and Morocco: 0.785).

The hierarchical AMOVA analysis showed that only a small and non-significant part of the genetic variance could be attributed to the variation between East and West groups \((F_{CT} = 0.30\%)\), indicating no particular genetic differentiation between both sides and arguing for a shared middle eastern ancestry. Botigué et al. have previously studied population relationships in the western and eastern Mediterranean basin using genome-wide SNP data from more than 2000 individuals. This survey indicated that Southwestern European population average between 4% and 20% of their genomes assigned to a North African ancestral whereas this value does not exceed 2% in southeastern European populations. They also found that migration event from North Africa to Europe would have occurred at least 6–10 generations ago \((\sim 240–300 \text{ ya})\) in Spain, and at least 5–7 generations ago in France and Italy. In the other hand, SNP data showed that the haplotype sharing between Europe and the Near East follows a southeast to southwest gradient, whereas sharing between Europe and the Maghreb follows the opposite pattern (Botigué et al., 2013; Henn et al., 2012); this suggests that gene flow from the Near East cannot account for the sharing with North Africa. It is possible that these patterns reflect more ancient migrations, perhaps dating back to the Neolithic, which resulted in a low level of short Near Eastern haplotypes across much of Europe. A model of gene flow from the Near East into both Europe and North Africa, such as a strong demic wave during the Neolithic, could result in shared haplotypes between Europe and North Africa. In other ways, studies focusing on mitochondrial DNA concluded that groups of the proto U6 lineage spread from the Near East to North Africa around 40–45 ka, followed by some degree of regional continuity. In Europe, U6 lineage was only found in the Iberian Peninsula, pointing likely to a northward expansion from Africa (Maca-Meyer et al., 2003; González et al., 2003). This expansion could be attributed to either the Arab–Berber occupation that lasted seven centuries or to prehistoric immigrations of North Africans to Iberia (Ibn Abd-el-Hakem, 1858) probably during the Caspian diffusion in North Africa and suggesting important demographic movements in the western area of this continent. For instance and in line with our results, it was found using classical genetic markers, that the Near East showed smaller genetic distances with East Africa than with West Africa (especially Arab groups) and Iberian Peninsula (Bentayebi et al., 2011; Botigué et al., 2013; Henn et al., 2012). Moreover, Cultural and religious changes within the last two millennia appear to have facilitated and maintained admixture between culturally similar populations from the Near East and North Africa. The deviation of Arabic Tunisian was of particular concern. This could be explained by the history of the Tunisian population, reflecting the influence of the ancient Phoenician settlers of Carthage followed, among others, by Roman, Byzantine, Arab and French occupations, according to historical records. Notwithstanding, other explanations cannot be discarded, such as the relative heterogeneity within current Tunisian populations, and/or the limited sub-Saharan genetic influence in this region as compared with other North African areas, without excluding the possibility of the genetic drift and the small size of the sampled population.

The total number of markers used in this study is quite small in comparison to many other available studies, but due to higher mutation rates and number of alleles per locus STRs provide much more information, on average, than SNPs for population assignment and population stratification (Listman et al., 2010). The fifteen used autosomal markers reveal a high population differentiation; this marker set may
be more useful for detecting recent admixture or founding events, such as those which formed the North African populations.

To sum up, our data on autosomal markers support, in general, the decreasing ancestry of the East-west populations described by other investigators (Henn et al., 2012; Maca-Meyer et al., 2003), providing, at the same time, detailed data of the frequency distribution of autosomal elements. These markers seem to perform well in fine-scale population differentiation studies. Also according to the statistical parameters the combined analysis of these 15 STR loci is a powerful tool for forensic identification and paternity testing in the Moroccan population.

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