Genetic variation at 5 new autosomal short tandem repeat markers (D10S1248, D22S1045, D2S441, D1S1656, D12S391) in a population-based sample from Maghreb region

**Aim** To investigate allele distribution and genetic parameters of a population-based sample from Maghreb region.

**Methods** Allele frequencies for 5 new autosomal short tandem repeat (STR) markers (D10S1248, D22S1045, D2S441, D1S1656, and D12S391) and several forensic parameters were determined for 95 unrelated individuals.

**Results** The combined power of discrimination and power of exclusion for the 5 loci were high (0.9999991 and 0.9954757, respectively). Allele frequencies were compared with previously published population data. Significant differences were found between Maghreb population and all other populations at the locus D2S441. Also, significant differences were found between the Maghreb and the African American population at the D22S1045, D1S1656, and D12S391 loci, between Maghreb and Caucasian population at the D1S1656 locus, and between Maghreb and Hispanic population at the D22S1045 locus.

**Conclusions** Typing of the 5 new STR loci may provide a useful addition to the previously established sets of autosomal STRs.
Short tandem repeats (STR) are widely used for forensic testing. Ordinary paternity cases are solved by commercially available multiplex kits, however, for more difficult cases, such as complex kinship analysis, additional STRs are needed to obtain better results. Besides, as many national DNA databases are growing and a large number of comparisons are being made within and between databases, concern for possible false-positive results may arise. This increases the need to introduce additional loci. The first European Standard Set (ESS) of loci included only 7 STRs loci, but the European Network of Forensic Science Institutes and the European DNA Profiling recommended to extend the ESS loci by adopting additional 3 miniSTRs loci (D10S1248, D22S1045, D2S441) and 2 additional polymorphic loci in 2006 (D1S1656, D12S391) (1,2).

These new 5 loci improve the discriminatory power of forensic analysis and, by amplifying fragments well below current average amplicon sizes, can enhance genotyping success when analyzing highly degraded DNA (3,4).

In order to verify and allow their use in forensics, the usefulness of ESS STR loci, it is necessary to obtain sufficient data from different populations.

METHODS

Saliva samples were obtained in 2010 from 95 unrelated, healthy immigrants from Maghreb region, whose both parents were born in Maghreb region (Morocco, Egypt, and Tunisia).

Genomic DNA was extracted from buccal swabs using Chelex® 100 method (Biorad, Richmond, CA, USA) (5).

PCR amplifications were performed in a GeneAmp® PCR System 9700 Gold Plate (Applied Biosystems, Foster City, CA, USA) using the commercial kit AmoFiSTR® NGM (Applied Biosystems), according to manufacturer’s recommendations (6). Typing was performed by capillary electrophoresis using an ABI Prism® 310 Genetic Analyzer (Applied Biosystems) and allele calling was performed with the software GeneMapperID V3.2 (Applied Biosystems), using manufacturer’s allelic ladders, bins, and panels.

For quality control, the laboratory regularly participates in the quality control proficiency testing programs provided by the GEDNAP group (German DNA Profiling, http://www.gednap.org).

Allele frequencies at each locus were calculated by direct counting. Statistical parameters of forensic interest were estimated: observed and expected heterozygosity (Hobs, Hexp) and standard error (7), polymorphism information content (8), power of discrimination (9), and power of exclusion (10,11).

### TABLE 1. Allele frequencies and statistical parameters for D10S1248, D22S1045, D2S441, D1S1656, and D12S391 short tandem repeat loci in a population sample from Maghreb region (n=95)*

| Locus        | Allele | Frequency | Hobs | Hexp | SE  | PIN | PD  | PE  |
|--------------|--------|-----------|------|------|-----|-----|-----|-----|
| D10S1248     | 0.0053 | 0.0053    | 0.0894 | 0.0263 | 10  |     |     |     |
|              | 0.1015 | 0.1263    | 0.3632 | 0.0579 | 11  |     |     |     |
|              |        | 0.0842    |       |      | 11.3|     |     |     |
| D22S1045     | 0.0053 | 0.0632    | 0.1526 | 0.0211 | 12  |     |     |     |
|              | 0.1026 | 0.1105    |       |      | 13  |     |     |     |
|              |        | 0.0526    |       |      | 13  |     |     |     |
| D2S441       | 0.1052 | 0.2789    | 0.0894 | 0.0053 | 14  |     |     |     |
|              |        | 0.0316    |       |      | 18  |     |     |     |
| D1S1656      | 0.3316 | 0.0263    | 0.1632 | 0.0263 | 15  |     |     |     |
|              |        | 0.0474    |       |      | 15  |     |     |     |
| D12S391      | 0.0105 | 0.1421    | 0.0105 | 0.0632 | 16  |     |     |     |
|              |        | 0.0211    |       |      | 16  |     |     |     |
|              |        | 0.0053    |       |      | 20  |     |     |     |

*Abbreviations: Hobs – observed heterozygosity; Hexp – expected heterozygosity; SE – standard error; HWE – P values from exact test for Hardy-Weinberg equilibrium; PIN – polymorphism information content; PD – power of discrimination; PE – power of exclusion.
clusion (10). ARLEQUIN software, version 3.11 (11) was used to assess deviations from Hardy-Weinberg equilibrium. Allele frequencies were compared with previously published population data using an exact test through the ARLEQUIN software, version 3.11 (11).

RESULTS AND DISCUSSION

A total of 95 samples were analyzed (Table 1). Deviation from Hardy-Weinberg equilibrium was detected for D22S1045 ($P = 0.0037$), D2S441 ($P = 0.0006$), and D12S391 ($P = 0.0002$) loci, even after a Bonferroni correction (12) for multiple testing ($P < 0.0100$). These deviations could be explained by an excess of homozygotes due to population substructure (Wahlund effect within the communities) or by a high inbreeding rate due to widespread endogamy (13,14). A larger sample size could help in understanding which of the two hypotheses is correct.

The combined power of discrimination and power of exclusion for the 5 new ESS STR loci were 0.9999991 and 0.9954757, respectively. Based on heterozygosity, D1S1656 may be considered the most informative locus ($H_{obs} = 0.8737$) and therefore especially useful in forensic investigations.

Allele frequencies for the 5 new ESS STR were compared with previously published population data (15-18) using an exact test and the ARLEQUIN software (11) (Table 2).

No significant differences were found from the already published data for the locus D10S1248; significant differences were found between Maghreb and the African American population at the D22S1045, D1S1656, and D12S391 loci, as well as between Maghreb and Hispanic population at the D22S1045 locus.

The obtained data demonstrate that these 5 new ESS STR loci are very useful for forensic purposes; the Maghreb population database could be helpful when testing individuals from this region.

Funding
None.

Ethical approval
Not required.

Declaration of authorship
VC was in charge of technical organization aspects of the study. NC was in charge of organization aspects of the study. AV was in charge of organization aspects of the study.

Competing interests
All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: no support from any organization for the submitted work; no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years; no other relationships or activities that could appear to have influenced the submitted work.

References

1 Gill P, Fereday L, Morling N, Schneider PM. The evolution of DNA databases – recommendations for new European STR loci. Forensic Sci Int. 2006;156:242-4. Medline:16002250 doi:10.1016/j.forsciint.2005.05.036

2 Gill P, Fereday L, Morling N, Schneider PM. New multiplexes for Europe-amendments and clarification of strategic development. Forensic Sci Int. 2006;163:155-7. Medline:16423481 doi:10.1016/j.forsciint.2005.11.025

3 Coble MD, Butler JM. Characterization of new miniSTR loci to aid analysis of degraded DNA. J Forensic Sci. 2005;50:43-53. Medline:15830996 doi:10.1520/JFS2004216

4 Phillips C, Fernandez-Formoso L, Garcia-Magariños M, Porras L, Tvedebrink T, Amigo J, et al. Analysis of global variability in 15 established and 5 new European Standard Set (ESS) STR loci. Forensic Sci Int Genet. 2011;5:155-69. Medline:20457091 doi:10.1016/j.fsigen.2010.02.003

5 Walsh PS, Metzger DA, Higuchi R. Chelex 100 as a medium for

| Compared population                  | D10S1248       | D22S1045       | D2S441         | D1S1656        | D12S391        |
|--------------------------------------|----------------|----------------|----------------|----------------|----------------|
| Italians (Northern Italy) (15)       | 0.16620 ± 0.0278 | 0.09550 ± 0.0205 | 0.00330 ± 0.0012 | 0.71785 ± 0.0216 | 0.12015 ± 0.0242 |
| Italians (Southern Italy) (16)       | 0.07545 ± 0.0198 | 0.31770 ± 0.0270 | 0.01630 ± 0.0077 | 0.93385 ± 0.0199 | 0.95185 ± 0.0107 |
| Polish (17)                          | 0.19565 ± 0.0392 | 0.51925 ± 0.0280 | 0.01200 ± 0.0044 | 0.22390 ± 0.0295 | 0.88705 ± 0.0164 |
| African Americans (18)              | 0.06760 ± 0.0221 | <0.0001        | 0.01630 ± 0.0014 | 0.00025 ± 0.0003 | 0.01295 ± 0.0055 |
| Caucasians (18)                     | 0.11240 ± 0.0256 | 0.06825 ± 0.0147 | 0.00025 ± 0.0003 | 0.01295 ± 0.0055 | 0.35415 ± 0.0428 |
| Hispanics (18)                      | 0.17650 ± 0.0164 | 0.02290 ± 0.0168 | <0.0001        | 0.16995 ± 0.0343 | 0.26690 ± 0.0342 |

*P – value of the exact test of population differentiation with 10 000 steps in the Markov chain length and 1000 steps of dememorization. In bold – significant differences ($P < 0.05$).
simple extraction of DNA for PCR-based typing from forensic material. Biotechniques. 1991;10:506-13. Medline:1867860

6 Applied Biosystem. AmpFISTR® NGM™ PCR Amplification Kit User's Manual. Foster City (CA): Applied Biosystem; 2009.

7 Nei M. Molecular evolutionary genetics. New York (NY): Columbia University Press; 1987.

8 Botstein D, White RL, Skolnick M, Davis RW. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet. 1980;32:314-31. Medline:6247908

9 Huston KA. Statistical analysis of STR data. Profiles DNA. 1998;1:14-5.

10 Ohno Y, Sebetan IM, Akaishi S. A simple method for calculating the probability of excluding paternity with any number of codominant alleles. Forensic Sci Int. 1982;19:93-8. Medline:7054064 doi:10.1016/0379-0738(82)90155-4

11 Excoffier L, Laval G, Schneider S. Arlequin, version 3.1: an integrated software package for population genetics data analysis. Bern (Switzerland): University of Bern; 2006.

12 Bland JM, Altman DG. Multiple significance tests: the Bonferroni method. BMJ. 1995;310:170. Medline:7833759

13 Yang RC. Genetic associations and multilocus statistics in a nonequilibrium diploid population. Genetics. 2000;155:1449-58. Medline:10880502

14 Overall AD, Nichols RA. A method for distinguishing consanguinity and population substructure using multilocus genotype data. Mol Biol Evol. 2001;18:2048-56. Medline:11606701

15 Cortellini V, Cerri N, Verzeletti A. Population data on 5 non-CODIS STR loci (D10S1248, D22S1045, D2S441, D15S1656, D12S391) in a population sample from Brescia county (Northern Italy). Forensic Sci Int Genet. 2011;5:e97-8. Medline:21269903 doi:10.1016/j.fsigen.2010.12.008

16 Barbaro A, Cormaci P, Votano S, Agostino A. Allele frequencies of the new European Standard Set (ESS) loci in a population of Southern Italy (Calabria). Forensic Sci Int Genet. 2011 Mar 11. [Epub ahead of print]. Medline:21398197 doi:10.1016/j.fsigen.2011.02.002

17 Parys-Proszek A, Kupiec T, Wolanska-Nowak P, Branicki W. Genetic variation of 15 autosomal STR loci in a population sample from Poland. Leg Med(Tokyo). 2010;12:246-8. Medline:20624686 doi:10.1016/j.legalmed.2010.05.002

18 Budowle B, Ge J, Chakraborty R, Eisenberg AJ, Green R, Mulero J, et al. Population genetic analyses of the NGM STR loci. Int J Legal Med. 2011;125:101-9. Medline:20878415 doi:10.1007/s00414-010-0516-7