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The 2011 GeFI collaborative exercise. Concordance study, proficiency testing and Italian population data on the new ENFSI/EDNAP loci D1S1656, D2S441, D10S1248, D12S391, D22S1045

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ABSTRACT

The 2011 collaborative exercise of the ISFG Italian Working Group GeFI was aimed at validating the five ENFSI/EDNAP miniSTR loci D1S1656, D2S441, D10S1248, D12S391 and D22S1045. The protocol required to type at least 50 multilocus profiles from locally resident individuals and two blind bloodstains in duplicate (i.e., using at least two different commercial kits), and to send the electropherograms to the Organizing Committee. Nineteen laboratories distributed across Italy participated, collecting a total of 960 samples. Full concordance was found for the five new miniSTRs as observed from the comparison of 13,150 alleles. The inspection of the electropherograms allowed the identification of a very limited number of mistypings in the miniSTR genotypes thus contributing to the establishment of an high quality Italian database of frequencies.

1. Introduction

The ISFG Italian Working Group GeFI plans periodical collaborative exercises aimed at the analysis of genetic markers relevant for the forensic application. In 2011, the Organizing Committee has proposed the characterization of the five new ENFSI/EDNAP loci, D1S1656, D2S441, D10S1248, D12S391 and D22S1045, in a large Italian population sample [1,2]. The inclusion of these miniSTR markers to the European Standard Set of loci (ESS) has been established by the Council Resolution 2009-C 296-01 of 30

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November 2009 on the exchange of DNA analysis results [3]. This decision was based on the results of collaborative exercises carried out by ENFSI and EDNAP groups which strongly suggested the characterization of these markers in order to improve the power of discrimination between individuals thus minimizing the possibility of adventitious matches in international DNA databases [4,5]. In addition, the miniSTR approach has been shown to be very useful in the analysis of degraded and modified DNA samples. The genetic results will be collected in order to build an Italian database of frequencies for these new markers useful for the following forensic applications. Moreover, different kits will be requested for the genetic typing in order to verify the concordance between genotypes. Finally, each participating laboratory will be asked to provide information on the processing of the samples (DNA extraction, quantification and typing) and the evaluation of the results, by means of a questionnaire.

2. Materials and methods

2.1. Samples

Nineteen laboratories participated in the collaborative exercise. Ten laboratories were located in North Italy (Turin, Genoa, Milan, Pavia, Brescia, Verona, Padua, Modena, Bologna, Ferrara), seven in Center Italy (Firenze, Pisa, Ancona, Terni, Rome) and two in South Italy (Bari).

Fifteen were forensic genetics labs, three medical genetics labs and one from a national criminal justice service. Each lab was requested to type at least 50 unrelated Italian individuals living in their area which provided the informed consent. Six labs selected blood samples, nine labs saliva samples and the remaining ones both blood and saliva.

2.2. DNA extraction

The labs reported different DNA extraction procedures in the questionnaire. The Qiagen columns (QIAamp DNA mini kit; Qiagen, Germany) and the phenol/chloroform extraction were the most followed (7 and 4 labs, respectively).

2.3. DNA quantification

Fourteen labs quantified the DNA extracts before STR analysis. Eight labs used UV-spectrophotometry and six labs qPCR protocols.

2.4. DNA amplification and concordance study

Each laboratory was requested to type their samples in duplicate, freely choosing a combination of at least two of the following kits: PowerPlex ESX, PowerPlex ESI (Promega, USA), AmpFlSTR NGM (Applied Biosystems, USA), and Investigator ESSplex, Hexaplex ESS (Qiagen, Germany). Six labs used in combination ESX+ESI, five NGM+ESSplex, three NGM+ESI, two NGM+ESX, one NGM+Hexaplex and two a combination of three or more kits. Each lab amplified DNA amounts varying from 0.2 to 1.3 ng following the manufacturers' recommendations.

2.5. DNA electrophoresis and analysis

The amplified products were run on ABI 310 (12 labs), 3130 (6 labs) and 3500 (1 lab) Genetic Analysers (Applied Biosystems). Most of the labs (16 out of 19) analyzed the electropherograms using the GeneMapper software (Applied Biosystems) even if three labs still used older analysis softwares (Genescan/Genotyper).

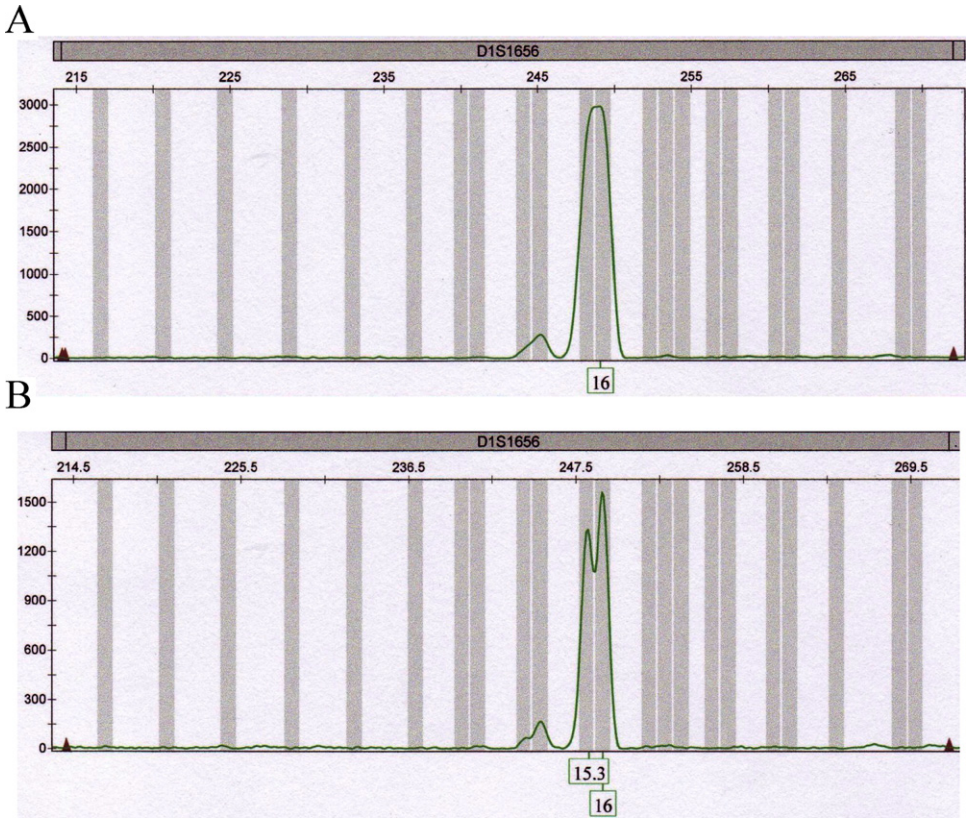

Fig. 1. Two electrophoretic separations of D1S1656 alleles 15.3 and 16. (A) Alleles not correctly separated and merged in allele 16. In this case, the number of runs performed by the capillary was close to the limit suggested by the manufacturer for the replacement (100 runs). (B) Once replaced, the same sample showed a correct separation and identification of alleles 15.3 and 16.

2.6. Lab certifications

Ten labs were certified UNI EN ISO 9001:2000 and one got the additional ISO 15189 certification. Eleven labs participate in GEDNAP quality control/proficiency tests, for forensic DNA typing certificates.

2.7. Proficiency testing

The Organizing Committee provided two blind bloodstains to each participating laboratory.

2.8. Statistical analyses

Statistical analyses were performed using Arlequin 3.0 [6].

2.9. Quality control

A result summary in the form an Excel file (tabular results) was sent to each participating laboratory to be filled with the genotypes found for each sample. In order to verify the quality and to confirm the results, each laboratory was requested to send all the electropherograms obtained from the samples to the Organizing Committee either as print outs or electronic files.

3. Results and discussion

The final database included the multilocus genetic profiles of 960 individuals, equally distributed among the 19 participating labs.

All the electropherograms were visually evaluated by two independent operators in order to verify the correctness of the genotypes assignments in the electropherograms and the correspondence in the tabular results.

Five laboratories experienced troubles in the electrophoretic separation of alleles for some new miniSTRs, using the ABI PRISM 310 sequencer. This is the case of alleles differing for 1 bp, typed for the D1S1656 and D12S391 markers, in the molecular range above 200 bp. This poor resolution resulted in false homozygote genotypes (see Fig. 1) and in apparent discordances in the genotypes when the same sample was characterized using a different kit showing a configuration of the same markers with amplicons below 200 bp. This problem was resolved either re-injecting the sample in order to get a better electrophoretic separation or optimizing the capillary eletrophoresis (CE) settings decreasing the injection time or adjusting the GeneMapper analysis parameter "peak detection".

As regards the concordance study, only two differences were observed out of a total number of 42,188 alleles inspected in the course of the exercise. One sample showed a null allele (18) for the vWA marker when amplified with NGM which was conversely heterozygote for ESSplex (17–18). The second sample exhibited the TH01 genotype 9–9.3 for ESSplex being homozygote 9–9 for NGM. All the dropouts were confirmed in a second amplification and CE. The rate of concordance between the NGM and ESSplex kits was thus calculated as 99.979% according to [7]. No discordance was recorded for the new miniSTRs in the Italian population sample here studied. Two discordances previously described in a preliminary study [8] were not confirmed by the reporting lab when contacted for revision.

The inspection of the electropherograms was mainly focused on the correct identification of the genotypes for the new miniSTRs, as one of the main aims of this collaborative exercise was to build an high quality Italian database of frequencies for those markers. This process allowed the identification of eight wrong genotypes for the D1S1656 and D12S391 markers which were correctly identified in a second amplification and CE when the labs were contacted for revising the samples in question. Eight genotypes for the new miniSTRs were "typos" or "clerical errors", that is mistakes in the course of the transcription of the tabular results.

In addition, six further samples displayed discordant genotypes in the duplicate analysis of D1S1656 and/or D12S391. This is the case, for instance, of a sample showing genotype 16–16 and 15.3–16, for the D1S1656 marker, when typed with the ESI and ESX kits, respectively. In case of discrepancies, the labs which performed the experiments arbitrarily decided to choose one genotype as the correct one thus reporting it in the tabular results. When the labs were contacted for revision, no more discordances were then observed and the correctness of the genotype assignment initially reported in the tabular results was confirmed.

On the opposite, after the revision of two different samples showing discordant genotypes for the D2S441 marker (genotypes 11–12 and 11–11.3, for ESI and ESX, respectively), a full concordance of genotypes was established but the genotypes initially reported in the tabular results were the wrong ones.

Hardy–Weinberg equilibrium was tested separately by sample ($N = 19$ samples) and by locus ($n = 5$ loci) with no evidence of deviation. A very low level of differentiation among samples was found by computing pairwise $F(ST)$ values and by partitioning Wright's F statistics, locus by locus (see Table 1).

Table 1
Allele frequencies distribution in the Italian population for the five miniSTRs D1S1656, D2S441, D10S1248, D12S391 and D22S1045.

Allele	D1S1656	D2S441	D10S1248	D12S391	D22S1045
8		0.001			
9		0.003	0.001		
10	0.002	0.140	0.001		0.001
10.3		0.001			
11	0.069	0.332	0.003		0.111
11.3		0.083	0.001		
12	0.140	0.039	0.027		0.005
12.3		0.002			
13	0.072	0.027	0.264		0.004
13.3		0.001			
14	0.091	0.320	0.328	0.001	0.049
14.3	0.002				
15	0.160	0.049	0.180	0.049	0.384
15.3	0.058				
16	0.130	0.003	0.154	0.019	0.352
16.3	0.047			0.001	
17	0.047		0.038	0.098	0.082
17.3	0.109			0.014	
18	0.007		0.005	0.197	0.009
18.3	0.053			0.034	
19	0.001		0.001	0.108	0.002
19.3	0.010			0.012	
20	0.001			0.122	
20.3	0.001			0.001	
21				0.107	
22				0.104	
23				0.074	
24				0.040	
25				0.014	
26				0.005	
27				0.001	
N	960	960	960	960	960
N_a	18	13	12	19	10
$N_a > 0.01$	12	7	6	14	5
H_{exp}	0.897	0.756	0.765	0.891	0.707
F_{IS}	−0.009	−0.047	−0.013	0.012	−0.012
MP	0.020	0.096	0.092	0.022	0.135
PE	0.791	0.544	0.547	0.781	0.467

N: number of typed individuals; N_a: number of alleles, $N_a > 0.01$: number of alleles with $p > 0.01$; H_{exp}: expected heterozygosity; F_{IS}: Wright's fixation index ($=1 - H_{obs}/H_{exp}$); MP: match probability; PE: probability of excluding a false father in standard trios.

Thus, allele frequencies were computed for all 960 typed subjects (see Table 1). The locus-by-locus exact tests of population differentiation were not significant of heterogeneity with previously published Italian data [9]; therefore, the conclusion of Welch [10], that population substructure in Europe can be accommodated using the correction factor FST = 0.01, can be extended to the present results.

All labs correctly typed the two blind blood stains provided by the organizers as proficiency testing.

The present GeFI collaborative exercise showed to be a very useful tool for monitoring the quality of the participating laboratory work. In fact, the double-check inspection of the electropherograms allowed us to identify a limited number of mistyping and ambiguous results otherwise hidden in a general population study, thus producing an high-quality database of frequencies.

The authors state that understand and accept the guidelines for publication of population data requested by the journal [11] and the International Society for Forensic Genetics (ISFG) [12].

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References

[1] M.V. Lareu, C. Pestoni, M. Schürenkamp, S. Rand, B. Brinkmann, A. Carracedo, A highly variable STR at the D12S391 locus, Int. J. Legal Med. 109 (3) (1996) 134–213.

[2] M.V. Lareu, S. Barral, A. Salas, C. Pestoni, A. Carracedo, Sequence variation of a hypervariable short tandem repeat at the D1S1656 locus, Int. J. Legal Med. 111 (5) (1998) 244–247.

[3] Council Resolution of 30 November 2009 on the exchange of DNA analysis results, http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:-C:2009:296:0001:0003:EN:PDF.

[4] P. Gill, L. Fereday, N. Morling, P.M. Schneider, The evolution of DNA databases – recommendations for new European STR loci, Forensic Sci. Int. 156 (2006) 242–244.

[5] V.C. Tucker, A.J. Hopwood, C.J. Sprecher, R.S. McLaren, D.R. Rabbach, M.G. Ensenberger, J.M. Thompson, D.R. Storts, Developmental validation of the PowerPlex® ESI 16 and PowerPlex® ESI 17 Systems: STR multiplexes for the new European standard, Forensic Sci. Int. Genet. 5 (2011) 436–448.

[6] L. Excoffier, G. Laval, S. Schneider, Arlequin ver. 3.0: an integrated software package for population genetics data analysis, Evol. Bioinform. Online 1 (2005) 47–50.

[7] C.R. Hill, D.L. Duewer, M.C. Kline, C.J. Sprecher, R.S. McLaren, D.R. Rabbach, B.E. Krenke, M.G. Ensenberger, P.M. Fulmer, D.R. Storts, J.M. Butler, Concordance and population studies along with stutter and peak height ratio analysis for the PowerPlex® ESX 17 and ESI 17 Systems, Forensic Sci. Int. Genet. 5 (2011) 269–275.

[8] C. Previderé, P. Grignani, S. Presciuttini, the GeFI Group, Italian population data for the new ENFSI/EDNAP loci D1S1656, D2S441, D10S1248, D12S391, D22S1045. The GeFI collaborative exercise and concordance study, Forensic Sci. Int. Genet. Suppl. Ser. 3 (2011) e238–e239.

[9] A. Berti, F. Brisighelli, A. Bosetti, E. Pilli, C. Trapani, V. Tullio, C. Franchi, G. Lago, C. Capelli, Allele frequencies of the new European Standard Set (ESS) loci in the Italian population, Forensic Sci. Int. Genet. 5 (2011) 548–549.

[10] L.A. Welch, P. Gill, C. Phillips, R. Ansell, N. Morling, W. Parson, J.U. Palo, I. Bastisch, European Network of Forensic Science Institutes (ENFSI): evaluation of new commercial STR multiplexes that include the European Standard Set (ESS) of markers, Forensic Sci. Int. Genet., http://dx.doi.org/10.1016/j.fsigen.2012.03.005, in press.

[11] A. Carracedo, J.M. Butler, L. Gusmao, W. Parson, L. Roewer, P.M. Scheider, Publication of population data for forensic purposes, Forensic Sci. Int. Genet. 4 (2010) 145–147.

[12] P.M. Schneider, Scientific standards for studies in forensic genetics, Forensic Sci. Int. 165 (2007) 238–243.