COLLABORATIVE STUDY ON THE POLYMORPHISM OF THE D1S80 LOCUS IN THE ITALIAN POPULATION.

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Summary.
Twenty Italian laboratories were involved in a collaborative study on the polymorphism of the locus D1S80. A total of 1720 blood samples of unrelated people collected in 19 Italian towns were analysed. The allelic frequencies reported by the participant laboratories were statistically homogeneous with the exception of one laboratory. More than 33 different alleles were found, ranging from 14 to over 41 repeats. Four interalleles were found and five alleles were larger than 41 repeats. A total of 146 genotypes was found. Phenotypic classes with expected frequency >4 were tested for Hardy-Weinberg equilibrium: the observed frequencies of the 51 classes did not differ significantly from the expected frequencies. No mutant allele was detected in 465 mother-child pairs.

Introduction.
Since the first appearance on the forensic scene of the use of DNA polymorphism analysis for personal identification and paternity testing there has been concern on a number of issues such as the reproducibility of the results, population differences and population stratification. In line with the recommendation of the EDNAP, the Italian Group of Forensic Haematologists (G.E.F.I.) launched a series of collaborative studies to standardise the techniques and to collect population data all over Italy. One of these studies concerned the polymorphic locus D1S80. This locus shows a large number of alleles and the collaborative study allowed the collection of a relatively large sample of the Italian population for the establishment of a reference data bank of allelic and genotypic frequencies.

Materials and methods.
Each participant laboratory was invited to collect and analyse a minimum of 50 blood samples obtained from residents in the corresponding region. The main characteristics of the common protocol adopted were: primers as reported by Kasai et al (1990), reference allelic ladder (T14-T40) and amplification conditions as for the commercial kit Cetus D1S80, allele separation and gel staining as reported by Budowle et al (1991). Some laboratories run the electrophoresis on agarose gels. Each participant was given a reference DNA with alleles widely separate to control possible preferential amplification and two bloodstains as a blind control.

Results.

Twenty laboratories took part in the collaborative exercise and a total of 1720 blood samples of unrelated people of 19 towns were analysed (Genova, Pavia, Brescia, Verona, Padova, Trieste, Parma, Modena, Ferrara, Bologna, Firenze, Pisa, Ancona, Camerino, Terni, Roma, Bari, Messina and Catania). The number of samples analysed by each laboratory ranged from 34 to 380. All laboratories correctly characterised the reference DNA and the two blind samples, although 4 laboratories gave no answer for the bloodstains.

Table 1.
Simplified allelic frequencies

Alleles	Obser.	%
14-18	680	20.97
19-21.5	166	5.12
22-23.5	231	7.13
24-24.5	1,227	37.85
25-26	201	6.20
27-29	422	13.02
30-42	315	9.72
Total	3,242	100

The allelic frequencies for each laboratory were computed and an homogeneity test among laboratories was performed. Because of the high number of alleles, the allelic frequencies were grouped into 7 allelic classes (see Table 1) to have the lowest expected frequency >4. The homogeneity test showed a highly significant variation among laboratories (X^2 =391.87 df=120 $p<0.01$). The pairwise comparison between laboratories showed that the majority of the variation was introduced by Laboratory 9 as reported in Table 2. This was probably due to laboratory 9 which changed the allelic ladder during the analyses and moreover included, (for a specific purpose of the laboratory), a population sample with 4 autochthonous grandparents. To avoid a possible bias, the data of this laboratory were excluded from further analyses. The remaining significant heterogeneity was scattered between laboratories and the allelic groups as would be expected for such a large comparison with large sample variation.

Table 2.
Pairwise comparisons between laboratories: X^2 test of the simplified allelic frequencies.

Lab.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	0																			
2	6.18	0																		
3	8.92	8.95	0																	
4	7.19	6.15	8.32	0																
5	6.71	3.75	10.3	5.55	0															
6	2.66	6.52	6.21	4.69	5.26	0														
7	**17.3**	11.6	**19.1**	7.26	10.4	3.49	0													
8	2.11	9.52	11.3	5.07	6.52	3.01	12.3	0												
9	**87.2**	**73.6**	**75.7**	**78.7**	**92.1**	**74.5**	**46.4**	**78.0**	0											
10	**14.7**	11.8	**17.9**	11.7	10.0	**13.8**	10.5	**13.7**	**59.9**	0										
11	9.95	**14.0**	6.67	10.4	9.17	6.40	**16.6**	8.37	**67.7**	11.2	0									
12	2.17	11.0	6.07	10.5	**13.4**	2.99	**21.3**	4.03	**87.6**	**19.1**	6.72	0								
13	4.16	6.33	8.35	1.48	3.87	3.49	12.0	3.07	**97.6**	**14.7**	8.75	7.28	0							
14	7.36	5.13	11.8	5.63	3.55	4.39	4.80	6.34	**48.0**	3.33	7.41	9.35	6.49	0						
15	9.58	8.49	8.98	3.42	9.65	7.46	**13.6**	7.42	**96.8**	6.56	5.78	9.96	5.27	4.85	0					
16	**13.1**	**13.1**	**18.7**	9.80	**12.7**	5.72	4.94	9.33	**82.3**	**14.3**	**16.5**	**15.4**	**13.9**	1.59	**18.4**	0				
17	10.0	6.81	9.55	2.03	5.37	7.41	9.72	7.84	**96.2**	**17.5**	**13.4**	**14.4**	2.71	9.10	8.80	**18.5**	0			
18	7.62	5.47	**15.6**	5.62	3.29	7.01	9.42	7.93	**45.1**	5.72	10.5	11.5	8.57	3.15	9.80	5.80	11.5	0		
19	8.97	6.95	**14.3**	5.44	6.08	6.42	3.37	7.77	**34.9**	7.62	11.0	12.3	6.78	4.73	7.92	4.51	7.20	6.77	0	
20	5.23	4.73	5.09	1.62	3.88	3.04	7.90	4.41	**69.1**	11.1	7.61	7.43	0.79	5.82	4.46	9.33	1.02	8.35	6.41	0

Bold values show a significant difference ($p<0.05$).

A total of 33 different alleles were observed (Table 3), ranging from 14 to over 41 repeats, notably four of these alleles dropped in between the rungs of the allelic ladder and they were provisionally designated with an interallelic value. Five alleles were larger than 41 repeats and they were all grouped in the 42 repeat class. The most frequent alleles were the 18 (20.4%) and the 24 (37,8%). As reported in other sudies, no 39 repeat allele was found: presumably this allele is either missing or very rare.

Of the 561 possible phenotypes only 146 were observed (Table 4). The most frequent phenotypes were: 18/24 (15.2%) and 24/24 (14.1%). The phenotypic classes with expected frequency >4 were tested for Hardy-Weinberg equilibrium: the 51 classes did not differ significantly from the expected frequencies (X^2=42.04, d.f.=31, P>0.05).

Table 3.
Allelic frequencies

Allele	Obs.	%
14	2	0.06
15	2	0.06
16	9	0.28
17	5	0.15
18	662	20.42
19	12	0.37
20	68	2.10
21	86	2.65
22	181	5.58
22.5	1	0.03
23	48	1.48
23.5	1	0.03
24	1226	37.82
24.5	1	0.03
25	137	4.23
26	64	1.97
27	42	1.30
28	172	5.31
29	208	6.42
30	49	1.51
31	154	4.75
31.5	1	0.03
32	29	0.89
33	9	0.28
34	13	0.40
35	4	0.12
36	15	0.46
37	20	0.62
38	7	0.22
39	0	0.00
40	8	0.25
41	1	0.03
> 41	5	0.15
Total	3,242	100

A total of 465 mother-child pairs was analysed to assess the mutation rate. No mutant allele was detected.

Discussion.

No new allele of the D1S80 genetic system was reported, in fact the interalles listed here were previously described by Schwartz et al (1994). Notably the allelic frequencies reported by the 19 participant laboratories were relatively homogeneous, suggesting a relative uniformity in the Italian population. Nevertheless we cannot exclude that there may be small allelic frequency variations in different regions. In fact there were wide differences in the number of samples and it is possible that more data could disclose small but statistically significant variations.

The allelic frequencies reported here are also very similar to those reported for other European populations (Advances in Forensic Haemogenetics, Vol.5; Skowasch et al, 1992) and for the American Caucasian population (Budowle et al, 1991). On one hand, this result confirms the D1S80 Caucasian population structure and, on the other, validates the results reported by the participant laboratories.

In conclusion, the success of the collaborative exercise allowed the establishment of a reference D1S80 data bank for the Italian population and proved that a reasonable standardisation of methods can be achieved.

References.

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Table 4.
Genotypic frequencies.

Alleles		Frequencies			X²
Lower	Upper	%	Obs.	Expcted	test
14	29	0.12	2	0.13	
15	18	0.06	1	0.41	
15	22	0.06	1	0.11	
16	16	0.06	1	0.01	
16	18	0.06	1	1.84	
16	20	0.06	1	0.19	
16	22	0.06	1	0.50	
16	24	0.12	2	3.40	
16	29	0.12	2	0.58	
17	22	0.06	1	0.28	
17	23	0.06	1	0.07	
17	24	0.12	2	1.89	
17	29	0.06	1	0.32	
18	18	5.18	84	67.59	3.98
18	20	0.86	14	13.89	0.00
18	21	1.17	19	17.56	0.12
18	22	1.97	32	36.96	0.67
18	22.5	0.06	1	0.20	
18	23	0.37	6	9.80	1.47
18	23.5	0.06	1	0.20	
18	24	15.18	246	250.3	0.08
18	25	1.79	29	27.97	0.04
18	26	0.62	10	13.07	0.72
18	27	0.37	6	8.58	0.77
18	28	1.85	30	35.12	0.75
18	29	2.53	41	42.47	0.05
18	30	0.56	9	10.01	0.10
18	31	1.67	27	31.45	0.63
18	32	0.43	7	5.92	0.20
18	33	0.12	2	1.84	
18	34	0.06	1	2.65	
18	35	0.12	2	0.82	
18	36	0.12	2	3.06	
18	37	0.25	4	4.08	0.00
18	40	0.06	1	1.63	
18	>41	0.06	1	1.02	
19	21	0.06	1	0.32	
19	24	0.37	6	4.54	0.47
19	25	0.06	1	0.51	
19	26	0.06	1	0.24	
19	27	0.06	1	0.16	
19	28	0.06	1	0.64	
19	29	0.06	1	0.77	
20	20	0.12	2	0.71	
20	22	0.25	4	3.80	
20	24	1.67	27	25.71	0.06
20	25	0.06	1	2.87	
20	26	0.19	3	1.34	
20	28	0.37	6	3.61	
20	29	0.25	4	4.36	0.03
20	31	0.12	2	3.23	
20	37	0.06	1	0.42	
20	40	0.06	1	0.17	
21	21	0.31	5	1.14	
21	22	0.19	3	4.80	0.68
21	23	0.19	3	1.27	
21	24	1.85	30	32.52	0.20
21	25	0.25	4	3.63	
21	26	0.06	1	1.70	
21	28	0.19	3	4.56	0.54
21	29	0.25	4	5.52	0.42
21	31	0.43	7	4.09	2.08
21	32	0.06	1	0.77	
22	22	0.56	9	5.05	3.08
22	23	0.12	2	2.68	
22	24	4.87	79	68.45	1.63
22	25	0.31	5	7.65	0.92
22	26	0.31	5	3.57	
22	27	0.25	4	2.34	
22	28	0.56	9	9.60	0.04
22	29	0.37	6	11.61	2.71
22	31	0.37	6	8.60	0.78
22	32	0.06	1	1.62	

Alleles		Frequencies			X²
Lower	Upper	%	Obs.	Expected	test
22	33	0.06	1	0.50	
22	34	0.12	2	0.73	
22	41	0.06	1	0.06	
23	24	1.11	18	18.15	0.00
23	25	0.19	3	2.03	
23	28	0.19	3	2.55	
23	29	0.19	3	3.08	
23	30	0.12	2	0.73	
23	31	0.19	3	2.28	
23	34	0.12	2	0.19	
23	37	0.06	1	0.30	
23	38	0.06	1	0.10	
24	24	14.07	228	231.8	0.06
24	25	2.96	48	51.81	0.28
24	26	1.67	27	24.20	0.32
24	27	1.05	17	15.88	0.08
24	28	4.07	66	65.04	0.01
24	29	5.12	83	78.66	0.24
24	30	1.36	22	18.53	0.65
24	31	3.39	55	58.24	0.18
24	32	0.74	12	10.97	0.10
24	33	0.31	5	3.40	
24	34	0.12	2	4.92	1.73
24	35	0.12	2	1.51	
24	36	0.62	10	5.67	3.30
24	37	0.43	7	7.56	0.04
24	40	0.25	4	3.03	
24	>41	0.06	1	1.89	
25	25	0.37	6	2.89	
25	26	0.06	1	2.70	
25	27	0.12	2	1.77	
25	28	0.31	5	7.27	0.71
25	29	0.62	10	8.79	0.17
25	30	0.19	3	2.07	
25	31	0.49	8	6.51	0.34
25	32	0.12	2	1.23	
25	36	0.12	2	0.63	
25	37	0.12	2	0.85	
26	26	0.06	1	0.63	
26	28	0.37	6	3.40	
26	29	0.19	3	4.11	0.30
26	30	0.12	2	0.97	
26	31	0.12	2	3.04	
26	37	0.06	1	0.39	
27	28	0.19	3	2.23	
27	29	0.12	2	2.69	
27	30	0.06	1	0.63	
27	31	0.25	4	2.00	
27	32	0.06	1	0.38	
27	38	0.06	1	0.09	
28	28	0.56	9	4.56	4.32
28	29	0.31	5	11.04	3.30
28	30	0.19	3	2.60	
28	31	0.43	7	8.17	0.17
28	32	0.06	1	1.54	
28	33	0.06	1	0.48	
28	37	0.12	2	1.06	
28	38	0.06	1	0.37	
28	>41	0.06	1	0.27	
29	29	0.56	9	6.67	0.81
29	30	0.25	4	3.14	
29	31	0.86	14	9.88	1.72
29	34	0.25	4	0.83	
29	36	0.06	1	0.96	
30	38	0.06	1	0.45	
30	32	0.19	3	0.44	
31	31	0.43	7	3.66	
31	32	0.06	1	1.38	
31	34	0.12	2	0.62	
32	32	0.12	2	0.13	
32	37	0.06	1	0.18	
38	38	0.12	2	0.01	
40	40	0.06	1	0.01	
	Total	100	1621	1621	42.04