TECHNICAL NOTE

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Allele and Genotype Frequencies for D1S80 and 3′APOB in Recanati, Central Italy*

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ABSTRACT: The VNTR 3′APOB and D1S80 loci were studied in a sample of 179 individuals living in the Recanati Area (Central Italy). For 3′APOB, we found 34 genotypes and 11 alleles. The system was in Hardy-Weinberg equilibrium. The observed and expected heterozygosity were 0.788 and 0.798 respectively. The discrimination power was 0.96, the a-priori paternity exclusion power was 0.619 and the polymorphism information content was 0.773. For D1S80, we found 45 genotypes and 18 alleles. The system deviated significantly from Hardy-Weinberg equilibrium. The observed and expected heterozygosity were 0.696 and 0.790 respectively. The discrimination power was 0.96, the a-priori paternity exclusion power was 0.617 and PIC was 0.767. The Recanati sample was compared with the general Italian frequencies for the 3′APOB locus. A difference of borderline significance was detected ($P = 0.04$). For D1S80, the sample was compared with a sample from Southern Italy and no significant difference was detected.

KEYWORDS: forensic science, DNA typing, population genetics, D1S80, 3′APOB, Recanati, Italy

The two VNTR loci used in this investigation, 3′APOB and D1S80, were described by Knott et al. in 1986 (1) and Nakamura et al. in 1988 (2), respectively. 3′APOB is located at the distal end of the short arm of chromosome 2 (2p24) immediately 3′ to the apolipoprotein B gene. Its repeat is 15–16 bp long (3) with two dominating sequences, type X and type Y, repeated from 25 to 55 times in the several alleles observed up to date (3,4). VNTR D1S80 is located on the short arm of chromosome 1 in telomeric position (1p35–p36) and its 16 bp repeat unit is repeated from 14 to 41 times and more in the alleles of the system (5).

In this paper, we report the allele and genotype frequencies at the 3′APOB and D1S80 locus in a sample of 179 unrelated individuals living in Recanati and its neighboring areas, in Central Italy, an area little known from the genetic viewpoint.

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Materials and Methods

The sample—The town of Recanati has 19 300 inhabitants and is located in the Region of Marche in Central Italy, on a hilly area not far from the Adriatic coast. Its population is relatively stable, it has grown from 13 000 to present size from 1861 to 1991, with a growth rate of 3 per thousand per year. Migration balance is −1.9 per thousand in 1992 (6). The present sample is made of 179 unrelated individuals born and resident in the Recanati area, who were referred to the Blood Bank of the Recanati General Hospital.

Alleles detection—Leukocytes were isolated from whole blood and DNA was extracted by salting out method (7) or by Chelex—100 method (8). Amplification was carried out under the following conditions: 3′APOB: temp. 94/58°C, time 1′,6′ for 26 cycles, primers suggested by Boerwinkle et al. (9); D1S80: temp. 94/65/72°C, time 1′,1′,3′ for 36 cycles, primers suggested by Kasai et al. (10).

Each mixture contained 125 ng of DNA for 3′APOB and 10 ng for D1S80, 10mM Tris-HCl (pH 9.0 at 25°C), 50 mM KCl, 1.5 mM $MgCl_2$, 1 μM each primer, 200 μM dNTPs and 2.5 μM Taq DNA polymerase (Promega, Madison). Amplified products were analyzed by electrophoresis in 2% agarose gel (NuSieve 3:1) stained with ethidium bromide, in TBE buffer at 70V for 18 h.

Reading gels was sometimes difficult, as it may happen in electrophoresis; this problem was solved once for all by scanning gels using the contrast enhancement option during image acquisition. The resulting file was then processed with the software Aldus Photostyler v. 2.0 and converted into a negative image with 3D effect (Fig. 1). The resulting images seem to eliminate any ambiguity in the reading of runs.

Statistics—The genotypes were identified and alleles were counted to test equilibrium by likelihood ratio. The level of significance of the likelihood ratios was ascertained by generating their empirical distribution by Monte Carlo methods. Moreover, we estimated the probability of matching (MP) (11), the discrimination power (DP) (12), the a-priori probability of exclusion (and the maximum probability of exclusion) of a given man in paternity cases (EP) (13), the polymorphism information content (PIC) (14). Finally, we tested Hardy-Weinberg following Chakraborty et al. (15).

Results and Discussion

Genetic typing results for 3′APOB and D1S80 are summarized in Table 1. For 3′APOB, 11 alleles and 34 genotypes were identi-

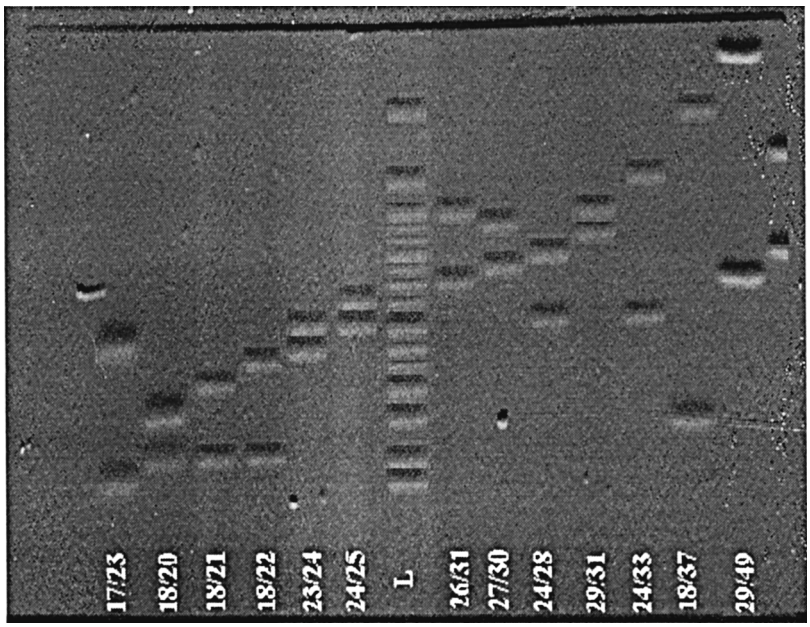

FIG. 1—*Polaroid photograph of a gel with ladder (above) and 3-D resolution of bands with the Aldus Photostyler software.*

TABLE 1—*Genotype frequencies at the 3'APOB and D1S80 loci in Recanati (*N *= 179).*

3'APOB						D1S80					
Genotypes	*N*	Freq.	Genotypes	*N*	Freq.	Genotypes	*N*	Freq.	Genotypes	*N*	Freq.
29–29	1	0.006	49–29	1	0.006	17–24	2	0.013	24–24	25	0.158
31–31	2	0.011	49–31	1	0.006	17–25	1	0.006	24–25	3	0.019
33–31	3	0.017	49–33	1	0.006	18–18	15	0.095	24–26	6	0.038
35–31	9	0.050	49–35	10	0.056	18–20	1	0.006	24–27	2	0.013
35–33	5	0.028	49–36	1	0.006	18–21	3	0.019	24–28	10	0.063
35–35	16	0.089	49–37	17	0.095	18–22	7	0.044	24–29	7	0.044
37–31	16	0.089	49–39	1	0.006	18–23	2	0.013	24–30	5	0.032
37–33	6	0.034	49–47	4	0.022	18–24	21	0.133	24–31	1	0.006
37–35	23	0.128	49–49	1	0.006	18–25	4	0.025	24–32	1	0.006
37–36	2	0.011	51–31	3	0.017	18–28	5	0.032	24–33	1	0.006
37–37	17	0.095	51–33	1	0.006	18–29	1	0.006	26–28	1	0.006
39–31	6	0.034	51–35	2	0.011	18–30	1	0.006	26–29	2	0.013
39–35	3	0.017	51–37	2	0.011	18–31	1	0.006	26–31	1	0.006
39–37	6	0.034	51–47	1	0.006	18–34	1	0.006	27–29	1	0.006
47–31	1	0.006	51–51	1	0.006	18–37	2	0.013	27–30	1	0.006
47–35	3	0.017	53–37	2	0.011	20–24	2	0.013	28–28	1	0.006
47–37	10	0.056				22–22	2	0.013	28–30	1	0.006
47–39	1	0.006				22–24	2	0.013	28–31	1	0.006
						22–25	1	0.006	29–29	2	0.013
						22–26	2	0.013	29–31	2	0.013
						23–23	2	0.013	29–34	1	0.006
						23–24	2	0.013	30–30	1	0.006
						23–25	2	0.013			
χ^2 (1df)*	0.071	$P = 0.790$				χ^2(1df)*	7.595	$P = 0.006$			
χ^2 (1df)†	0.266	$P = 0.606$				χ^2 (1df)†	2.477	$P = 0.118$			
G^2(23 df)	60.956	$P = 0.110$				G^2(27 df)	115.197	$P = 0.017$			
H obs		0.788				H obs		0.696			
H exp		0.798				H exp		0.790			
MP		0.041				MP		0.044			
DP		0.959				DP		0.956			
EP		0.619				EP		0.617			
PIC		0.773				PIC		0.767			

* Chi Square test based on observed/expected homozygotes and heterozygotes individuals.
† Chi Square test based on observed/expected homozygotes and heterozygotes genotypes.

TABLE 2—*Comparison of alleles frequencies at the 3'APOB locus and D1S80 in the Recanati population and other Italian populations.*

3'APOB			D1S80		
Allele	Recanati	Italy	Allele	Recanati	Southern Italy
31	43	250	18	79	32
33	16	181	22	16	11
35	87	583	24	115	90
37	118	761	25	11	6
39	17	114	26	12	6
47	20	157	28	20	22
49	38	150	29	18	10
Others	19	166	31	6	9
Total	358	2362	Other	39	20
			Total	316	206
χ^2 (7 df) = 15.109 $P < 0.04$			χ^2 (8 df) = 13.882 n.s.		
G^2 (7 df) = 14.826 $P < 0.04$			G^2 (8 df) = 13.634 n.s.		

fied; two alleles, 35 and 37, accounted for more than half the alleles in the population pool. Observed 3′APOB allele frequencies conform to Hardy-Weinberg equilibrium expectations (Table 1).

D1S80 typing showed 18 alleles and 45 genotypes; alleles 18 and 24 accounted for just over 60% of the alleles in this population pool. D1S80 in the Recanati population appears to deviate from Hardy-Weinberg equilibrium expectations. In the statistical analysis of VNTR systems, testing Hardy-Weinberg equilibrium requires three main steps: 1) the comparison of observed and expected heterozygosity; 2) the comparison of the number of observed and expected homozygous and heterozygous genotypes; and 3) the computation of the likelihood ratio G^2.

For D1S80, the G^2 is significant after 3000 bootstraps, due to the presence of rare homozygotes. The chi square of homozygotes versus heterozygotes is 7.595, with $P < 0.006$. Only the number of distinct homozygous and heterozygous genotypes is consistent with expectation. However, with these numbers of alleles and with these sample size caution is necessary in drawing conclusions about equilibrium. Moreover, other Italian samples show no deviation from Hardy-Weinberg equilibrium (16,17).

Heterozygosities, a priori match probabilities, discriminant power, paternity exclusion probabilities and PIC values are also shown in Table 1; the two polymorphic loci exhibit comparable values.

Table 2 compares allele frequencies in the Recanati population sample to frequencies observed in other Italian populations. For 3′APOB, frequency differences for alleles 33 and 49 between the Recanati and a general Italian population resulted in borderline statistical significance. For D1S80, the comparison of the allele frequencies with those of a sample from southern Italy (18) did not show significant differences.

Conclusion

In our study, 11 alleles were identified at the 3′APOB locus and 18 at the D1S80 locus. Both loci are highly polymorphic. The genotype frequencies of the major alleles are consistent with Hardy Weinberg equilibrium. With the given number of observed alleles, 34 of the 66 possible genotypes for 3′APOB and 45 of the 171 possible genotypes for D1S80 were detected.

The considerable discrimination power of both loci confirms their usefulness in forensic medicine, and their large PIC suggests that they be used as primary markers in linkage studies.

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