The evolution of DNA typing in the forensic haemogenetics laboratory of Parma

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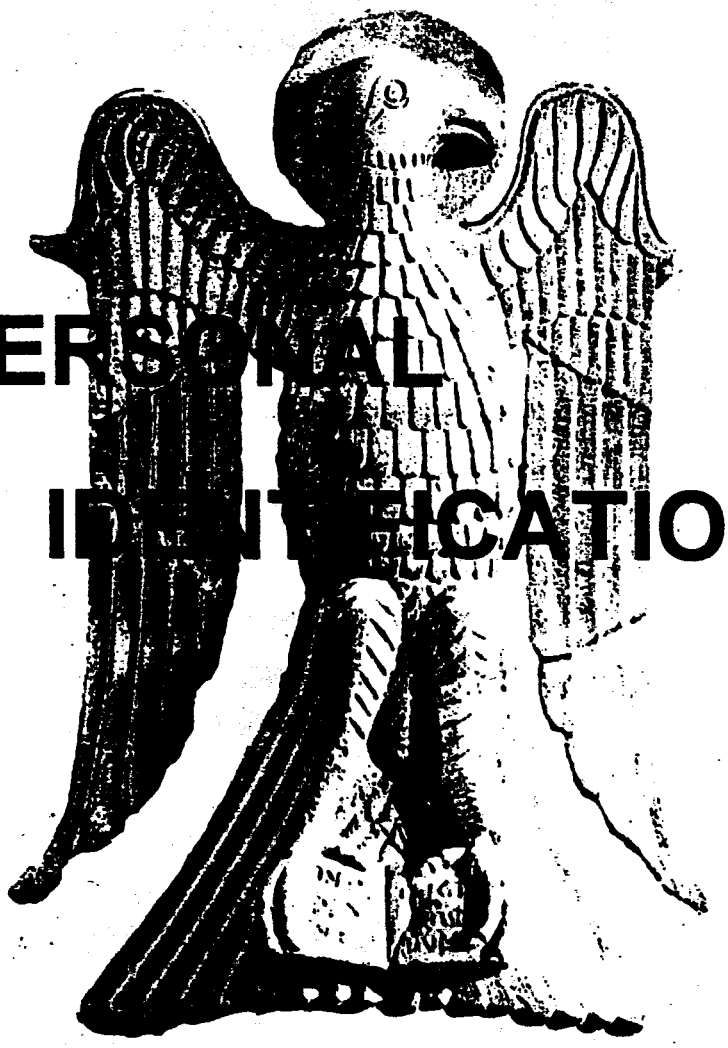

PERSONAL IDENTIFICATION

PROCEEDINGS

THE EVOLUTION OF DNA TYPING IN THE FORENSIC HAEMOGENETICS LABORATORY OF PARMA

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In the last few years, after the discovery of VNTR systems (Nakamura et al., 1987) and HLA class II sequence polymorphism (Saiki et al., 1986), the analysis of DNA polymorphisms has been routinely introduced into the leading Forensic Haemogenetics laboratories, both in Italy and abroad.

DNA Typing is no longer considered just an experimental technique but has become a powerful tool in forensic science both for paternity testing and criminal identification.

The Forensic Haemogenetics laboratory of Parma, in close collaboration with that of Ancona has undertaken various population studies over the last two years to determine the allelic distribution of various DNA polymorphisms and to assess their technical reliability and information yield in identity testing.

Research programs have accurately followed the progresses made in DNA studies, concentrating particularly on PCR-based DNA polymorphisms (Saiki et al., 1985). A sequence polymorphism (HLA DQ- α) (Saiki et al., 1986) was analysed initially, followed by length polymorphisms (AMP-FLP's and STR's) such as YNZ22, 3'Apo-B, MCT118, COL2A1, HUMTH01, HUMvWA31. More recent undertakings have included a preliminary investigation into the MVR-PCR technique, whose DNA typing potential appears to make it the most powerful tool in forensic identification so far achieved.

The present work shows the allelic frequency distribution of the PCR polymorphisms studied and their efficiency in forensic

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Materials and Methods

700 µl blood samples drawn from healthy blood donors, living in the Province of Parma, have been used in all population studies. DNA was extracted with Phenol-Chloroform technique (Budowle et al., 1990). 40 individuals were studied for DQ-α polymorphism, 115 for 3'Apo-B, 89 for MCT118, 100 for YNZ22, 103 for COL2A1, 146 for HUMTH01, and 97 for HUMvWA31.

Amplification was performed according to Cetus-Perkin Elmer protocols, Boerwinkle et al (1989), Kasai et al. (1990), Rand et al. (1992), Rand et al. (1992), Wiegand et al (1993a), and Wiegand et al (1993b).

Reverse Dot Blot Hybridization was performed for allelic identification of the DQ- α system, whereas AMP-FLP and STR alleles were identified by means of electrophoretic separation, either in agarose gel or high-resolution PAGE (Budowle et al, 1991) using a discontinuous buffer (Allen et al., 1989).

In all cases allele size identification was measured by comparison with molecular size markers (123 bp ladder, pGem ladder, X174 ladder) using the local reciprocal method of Elder and Southern (1983) (YNZ22, 3'Apo-B, MCT118) or by comparison with home-made allelic ladders (COL2A1, HUMTH01, HUMvWA31).

The results were analysed to determine the heterozygosity rate, the chance of exclusion (CE) calculated using the equation of Garber and Morris (1983) and the discrimination index (DI) according to Sensabaugh (1982).

MVR-PCR (Cucurachi et al., 1994) was performed on 60 individuals; amplified products were electrophoretically separated and analysed by Southern Blotting (Southern, 1975).

Results and Conclusions

The polymorphic markers studied have shown a relatively high number of alleles (from 6 for DQ-α and HUMTH01 to 19 for MCT118), and consequently a high heterozygosity rate and forensic efficiency.

The allele distribution of both sequence and length polymorphism is shown in Tables 1-3. Table 4 shows the heterozygosity rate and the single and combined values of mean CE and the DI for all PCR systems investigated.

Table 1: DQ-α allele frequencies

DQ-α	Allele					
	DQA1*0101	DQA1*0102	DQA1*0103	DQA1*0201	DQA1*0301	DQA1*0401
Frequency	0.113	0.2	0.088	0.15	0.063	0.388

Table 2: Allele frequencies of four AMP-FLP's

3'ApoB		MCT118		YNZ22		COL2A1	
Allele	Frequency	Allele	Frequency	Allele	Frequency	Allele	Frequency
29	-	16	0.017	1	0.047	1.1	0.004
31	0.091	17	-	2	0.202	1	0.102
33	0.061	18	0.225	3	0.181	2	-
35	0.235	19	0.039	4	0.262	3	-
36	0.004	20	0.022	5	0.043	4	-
37	0.409	21	0.034	6	0.017	5	0.233
39	0.052	22	0.045	7	0.013	6	0.252
41	-	23	0.005	8	0.017	7	0.029
43	0.004	24	0.326	9	0.103	8	-
45	-	25	0.067	10	0.065	9	-
47	0.056	26	0.011	11	0.004	10	0.335
49	0.078	27	0.011	12	0.034	11	-
51	0.008	28	0.015	13	0.004	12	0.043
		29	0.056	14	-	13	-
		30	0.005	15	0.004	14	-
		31	0.045			15	-
		32	0.017				
		33	0.005				
		34	0.005				
		35	-				
		36	-				
		37	0.017				
		38	-				

Table 3: Allele frequencies of 2 STR's

HUMTH01		HUMvWA31	
Allele	Frequency	Allele	Frequency
6	0.325	5	0.005
7	0.123	8	0.077
8	0.110	9	0.123
9	0.164	10	0.175
10	0.267	11	0.314
11	0.010	12	0.221
		13	0.072
		14	0.010

Table 4: Heterozigosity rate and single and combined mean CE and DI for 7 PCR systems

	Heterozygosity	Mean CE	DI
DQ-α	0.87	0.567	0.1
3'ApoB	0.79	0.556	0.098
MCT118	0.80	0.691	0.080
YNZ22	0.79	0.679	0.053
COL2A1	0.74	0.533	0.101
HUMTH01	0.74	0.553	0.088
HUMvWA31	0.81	0.601	0.066
COMBINED		0.998	$1.73*10^{-8}$

Highly polymorphic diploid codes have been generated by Minisatellite Variant Repeat analysis (MVR-PCR). Any of the individuals studied could be distinguished from any other using only the first nine repeat units and showed a mean number of 25 mismatches.

The results achieved so far in the Forensic Haemogenetics laboratory of Parma, underlining the efficiency and high informative power of PCR-based polymorphisms, reinforce our decision to extend the research to biological stains analysis and new STR's systems investigated.

References

- Allen R.C., Graves G., Budowle B. (1989) Polymerase chain reaction amplification products separated on rehydratable polyacrylamide gels and stained with silver. Bio. Tech. 7:736-744

- Boerwinkle E., Xiong W., Fourest E., Chang L. (1989) Rapid typing of tandemly repeated hypervariable loci by the polymerase chain reaction: application to the apolipoprotein B 3' hypervariable region. Proc. Natl. Acad. SCI. USA 86:212-216

- Budowle B., Baechtel F.S. (1990) Modification to improve the effectiveness of restriction fragment length polymorphism typing. Appl. Theor. Elettrophor., 1, 181

- Budowle B., Chakraborty R., Giusti A.M., Eisenberg A.J., Allen R.C. (1991) Analysis of the variable number of tandem repeats locus D1S80 by the polymerase chain reac-tion followed by high resolution polyacrylamide gel electro-phoresis. Am. J. Hum. Genet. 48:137-144

- Cucurachi N., McKeown B., Tagliabracci A:, Buscemi L., Masotti G., Ferrara S.D. (1994) Minisatellite Variant Repeat (MVR-PCR) nella identificazione personale. Riv. It. Med. Leg. (in press)

- Garber R.A., Morris J.W. (1983) General equations for the average power of exclusion for genetic system of n codominant alleles in one-parent and no-parent cases of disputed parentage. In: Walker R.H. (ed) inclusion proba-bilities in parentage testing. AABB, Arlington, VA, pp. 277-280

- Kasai K., Nakamura Y., White R.L. (1990) Amplification of a variable number of tandem repeats (VNTR) locus (pMCT118) by the polymerase chain reaction (PCR) and its application to forensic science. J. Forensic Sci. 35:1196-1200

- Nakamura Y., Leppert M., O'Connell P., Wolff R., Holm T., Culver M., Martin C., Fujimoto E., Hoff M., Kumlin E., White R. (1987) Variable number of tandem repeat (VNTR) markers for human gene mapping. Science 235: 1616-1622

- Rand S., Puers C., Skowasch K., Wiegand P. Budowle B., Brinkmann B. (1992) Population genetics and forensic efficiency data of 4 AMPFLP's. Int. J. Leg. Med. 104:329-333

- Saiki R.K., Bugawan T.L., Horn G.T., Mullis K.B., Erlich H.A., (1986) Analysis of enzimatically amplified betaglobin and HLA-DQ DNA with allele-specific oligonucleotide probes. Nature 324:163-166

- Saiki R.K., Scharf S., Faloona F., Mullis K.B., Horn G.T., Erlich H.A., Arnheim N. (1985) Enzymatic amplification of betaglobin genomic sequences

and restriction analysis for diagnosis of sickle cell anemia. Science 230:1350-1354

- Sensabaugh G.F. (1982) Biochemical markers of individuality. In: Saferstein R. (ed) Forensic Science Hand-book, Prentice Hall, Englewood Cliffs, pp. 340-415

- Southern E.M. (1975), Detection of specific sequences among DNA fragments separated by gel electrophoresis, J. Mol. Biol. 98:503-527

- Wiegand P., Budowle B., Rand S., Brinkmann B. (1993a) Forensic validation of the STR systems SE33 and TC11. Int. J. Leg. Med. 105:315-320

- Wiegand P., Bajanowsky T., Brinkmann B. (1993b) DNA typing of debris from fingernails. Int. J. Leg. Med. 106:81-83