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pMCT 118 (D1S80): a new allelic ladder and an improved electrophoretic separation lead to the demonstration of 28 alleles

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Summary. Population data studies carried out on caucasians from Northwest Germany ($n = 218$) using the AMPFLP system pMCT 118 (D1S80). The method used in a previous study (Rand et al. 1992) for pMCT 118 could be improved by increasing the electrophoretic separation length from 10 to 20 cm and by using an extended allelic ladder which allowed the distinction of 8 additional alleles (a total of 28 alleles). Out of the 8 additional alleles 5 could be differentiated which differed within the 16 bp repeat sequence. The allele frequencies found were compared to population data from American caucasians, Hispanics and black Americans (Eisenberg and Maha 1991). All populations with the exception or black Americans, showed good agreement.

Key words: AMPFLP-system pMCT 118 – Allelic ladder – Comparison of population data

Zusammenfassung. Eine Populationsstudie an nordwestdeutschen Kaukasiern ($n = 218$) wurde mit dem AMPFLP-System pMCT 118 (D1S80) durchgeführt. Als Modifikation zu einer zuvor durchgeführten Populationsstudie (Rand et al. 1992) wurde die elektrophoretische Trennstrecke von 10 cm auf 20 cm verlängert und ein erweiterter Allelstandard verwendet, wodurch 8 weitere (insgesamt 28) Allele unterschieden werden konnten. Innerhalb der 16 bp Repeat-Sequenz ließen sich 5 „Zwischenallele" diskriminieren. Die erhaltenen Allelfrequenzen wurden mit Allelfrequenzen amerikanischer Kaukasier, „hispanics" und schwarzen Nordamerikanern (Eisenberg und Maha 1991) verglichen. Abgesehen von den Populationsdaten schwarzer Nordamerikaner zeigte sich eine gute Übereinstimmung im Frequenzprofil.

Schlüsselwörter: AMPFLP-System pMCT 118 – Allelfrequenzen – Populationsvergleich

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Introduction

The present investigation on pMCT 118 (D1S80) is a continuation of other studies (Kasai et al. 1990; Budowle et al. 1991; Rand et al. 1992; Skowasch et al. 1992) with the following aims:

- comparison of an improved allelic ladder and nomenclature (Budowle 1992, personal communication) with the allelic ladder used previously (Budowle et al. 1991; Rand et al. 1992)
- more precise definiton of the alleles using an increased electrophoretic separation length
- comparison to other population data
- to examine whether new variants could be found with varying repeat length.

Material and methods

DNA from related and unrelated individuals was extracted from blood as previously described (Brinkmann et al. 1991a). Amplification (Rand et al. 1992) of the D1S80-locus was carried out using 80 ng template DNA and the primers described by Budowle et al. (1991).

Temperature profile: Denaturation 95°C/60 s; annealing 65°C/60 s; extension 72°C/480 s: 25 cycles with Triothermoblock (Biometra, FRG).

Separation of the fragments was carried out by discontinuous gel electrophoresis (Allen et al. 1989):

- Polyacrylamide gels: 6% T, 3% C, thickness 750 µm with piperazine diacrylamide (0.18% final concentration) as crosslinker and 60 m*M* Tris formate buffer
- Agarose plugs: 2% agarose with 0.52 *M* Tris and 0.28 *M* borate – separation length (bromophenol blue marker): 20 cm
- running time: approx. 4 h at 600 V, 15 mA, 10 W (Budowle 1992, personal communication).

The amplified fragments were visualised by silver staining (Budowle et al. 1991). The improved allelic ladder consisted of 20 alleles differing as a rule by 1 repeat (Fig. 1). Phenotyping was made by side-to-side comparison.

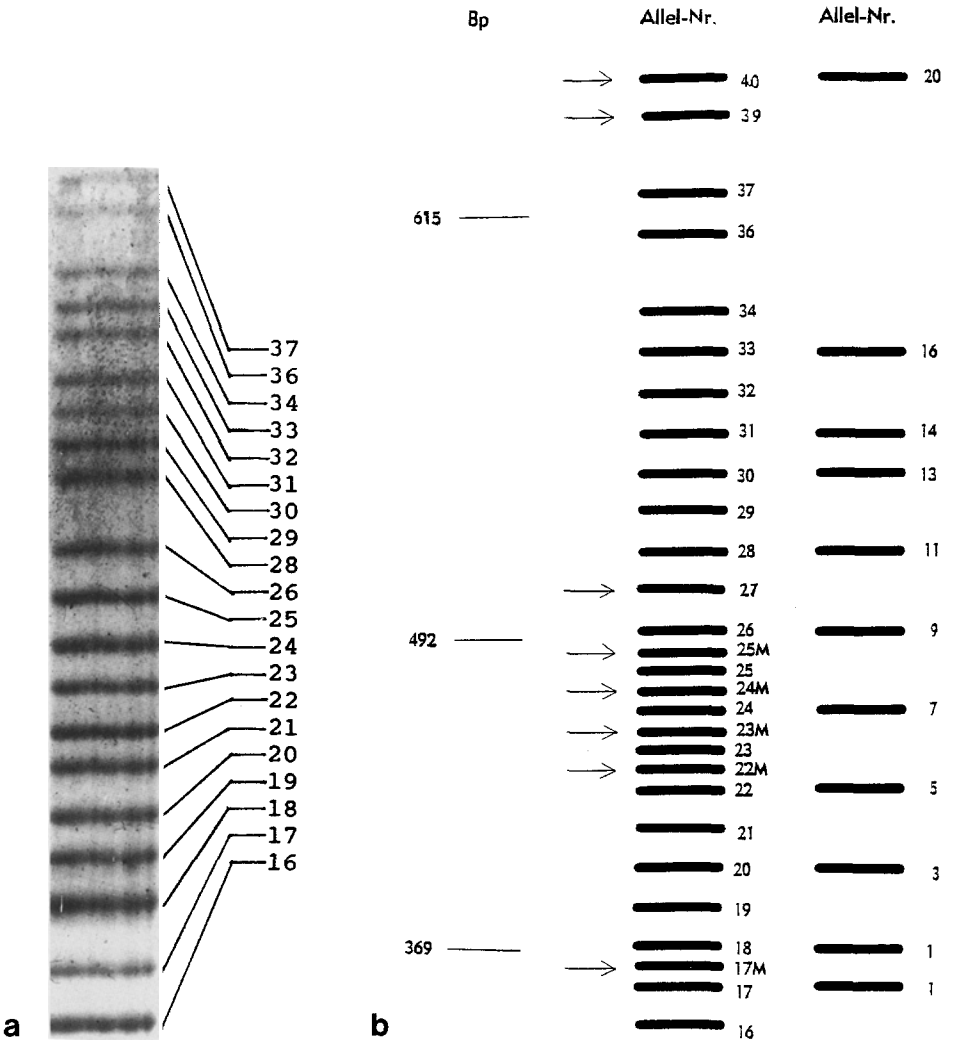

Fig. 1. a Improved allelic ladder for pMCT 118. The numbers are consistent with the number of repeats. Fragments 35 and 27 are not included in the ladder. **b** Comparison of the previous allelic ladder *(right)* with the new ladder and nomenclature *(left)* with the addition of 8 alleles (indicated by an *arrow*) – 5 "interalleles" (marked by "*M*") and 3 further alleles (which correspond to the 16 bp repeat spacing) found in this study

Results

In this population study ($n = 218$ individuals) 28 alleles (Table 1) could be distinguished. In addition to the expected fragments with multiples of the 16 bp repeat, 5 "interalleles" were diagnosed (Figs. 1b, 2) and 3 further alleles were found which were not present in the allelic ladder (Fig. 1). The alleles 18 ($f = 24.5\%$) and 24 ($f = 36.7\%$) were most common (Table 1) which is in agreement with previous studies (Rand et al. 1992; Skowasch et al. 1992).

The use of the improved allelic ladder and the optimization of fragment separation led to a 10% increase in the mean exclusion chance (AVACH, Krüger et al. 1968), the discrimination index could be improved by 80% and the heterozygosity rate by 10% (Table 2).

Hardy-Weinberg equilibrium

The proof that a population conforms with the Hardy-Weinberg rule can only be carried out for relatively small sample surveys using a modified procedure because of the large number of possible phenotypes (theoretical number of phenotypes in this study = 406). By forming groups of alleles or bins (Brenner and Morris 1990), each with adequate frequencies (Rand et al. 1992) the resulting phenotype frequencies can be calculated. No signifiant deviations from Hardy-Weinberg equilibrium could be found (Table 3).

Comparison of different population data

A qualitative comparison between different populations showed that allele frequencies were quite similar (Fig. 3) and the most common alleles showed especially (alleles 18 and 24) good agreement.

Obvious differences exist by comparison with the black population (Fig. 3), where 3 peaks of similar height (alleles 21, 24, 28) represent 50% of the alleles found.

In this study 75 families have also been investigated with a total of 75 offspring (150 parent/child meioses). The families had also been confirmed using conventional blood group systems and no evidence of incompatibility or new mutations was found.

Table 1. Allele designation and frequencies (present study)

Allele frequency	*n*	%
Allele		
16	1	0.2
17	3	0.7
17M	1	0.2
18	107	24.5
19	1	0.2
20	16	3.7
21	8	1.8
22	13	3
22M	2	0.5
23	7	1.6
23M	2	0.5
24	160	36.7
24M	5	1.1
25	24	5
25M	1	0.2
26	3	0.7
27	3	0.7
28	26	6
29	17	3.9
30	3	0.7
31	21	4.8
32	0	0
33	1	0.2
34	4	0.9
35	0	0
36	1	0.2
37	4	0.9
38	0	0
39	1	0.2
40	1	0.2
	436 alleles	

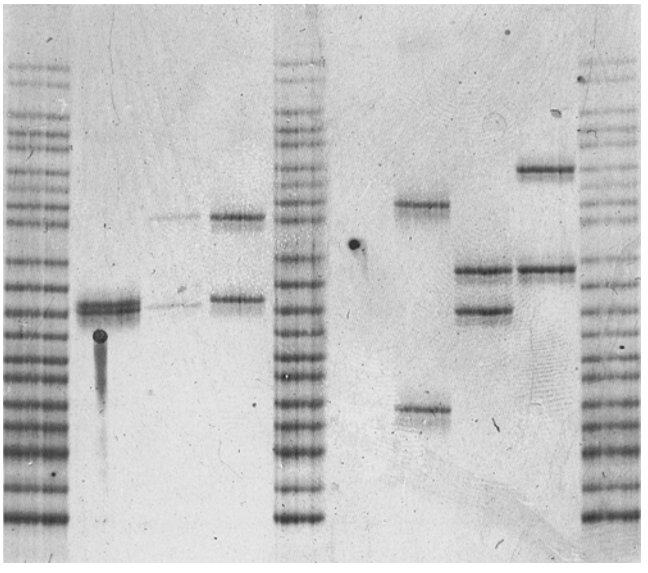

Fig. 2. Pherogram of the amplified fragments of 2 families. Allelic ladder = lanes 1, 5, 10; child = lanes 3, 8; mother = lanes 4, 9; putative father = lanes 2, 7 (excluded pf); "interalleles" = lanes 2, 4, 8, 9

Table 2. Comparison of efficiency data obtained using 2 different allelic ladders

	Population – study I (Rand et al. 1992)	Population – study II (present study)
Mean exc. chance	0.58	0.62
Disc. index	0.11	0.065
Heterozygosity	0.75	0.81

Table 3. Check for Hardy-Weinberg equilibrium (for details see text)

	4 allele model	3 allele model
Allele group		
I	Alleles 16–18	Alleles 18–23
II	Alleles 19–23	Allele 24
III	Allele 24	Alleles 25–40, 16, 17
IV	Alleles 25–40	
Chi^2	6.08	5.80
P	0.7–0.8 ($df = 9$)	0.3–0.4 ($df = 5$)

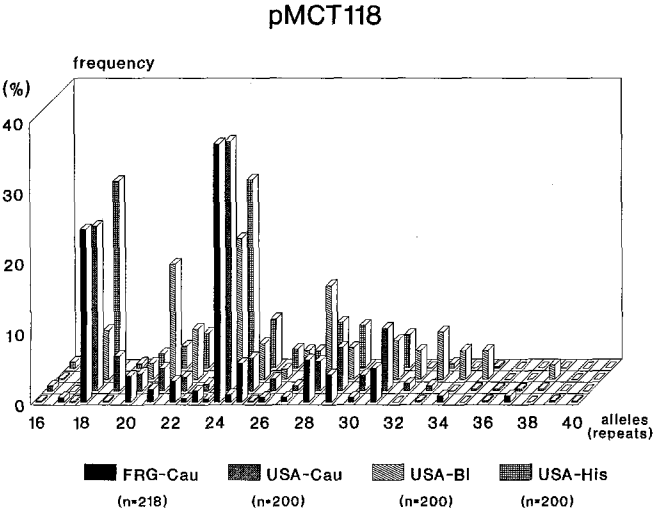

Fig. 3. Comparison of different population studies from Eisenberg and Maha (1991) and the present study. The nomenclature of Eisenberg and Maha (1991) has been aligned to the nomenclature used in the present study. FRG-Cau = German caucasians; USA-Cau = American caucasians; USA-Bl = black Americans; USA-His = hispanic Americans

Discussion

The results of sequencing at the D1S80 locus carried out by Kasai et al. (1990) showed that there is a stable 16 bp repeat and this is, for the most part, substantiated by the present study. But in addition it was noticed that improved separation conditions led to the distinction of some "interalleles".

The good agreement found between the European populations suggests a homogeneity within these caucasians. The clear difference to the black population dem-

onstrates a phenomenon which has been known for a long time in conventional haemogenetics. For hypervariable RFLP's (MS8, MS31 – Wong et al. 1987; YNH24 – Nakamura et al. 1987) the forensic significance of differences in frequency between different races and subpopulations has also been pointed out (Brinkman et al. 1991a, b; Lander 1991; Lewontin and Hartl 1991). This could lead to substantial differences in calculated frequency for a DNA pattern of an individual (Lander 1989) depending on the data base used.

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References

Allen RC, Graves G, Budowle B (1989) Polymerase chain reaction amplification products separated on rehydratable polyacrylamide gels and stained with silver. Biotechniques 7:736–744

Brenner C, Morris JW (1990) Paternity index calculations in single locus hypervariable DNA probes: validation and other studies. In: Proceedings for the International Symposium on Human Identification. Promega Corporation, Madison, USA, pp 21–541

Brinkmann B, Rand S, Wiegand P (1991a) Population and family data of RFLP's using selected single- and multi-locus systems. Int J Leg Med 104:81–86

Brinkmann B, Wiegand P, Rand S (1991b) A critical review of the forensic application of DNA technology. In: Berghaus G, Brinkmann B, Rittner C, Staak M (eds) DNA Technology and its forensic application. Springer, Berlin Heidelberg NewYork, pp 41–49

Budowle B, Chakraborty R, Giusti AM, Eisenberg AJ, Allen RC (1991) Analysis of the variable number of tandem repeats locus D1S80 by the polymerase chain reaction followed by high resolution polyacrylamide gel electrophoresis. Am J Hum Genet 48:137–144

Eisenberg M, Maha G (1991) AMPFLP analysis in parentage testing. In: Proceedings from the Second International Symposium on Human Identification, Promega Corporation, Madison, USA, pp 129–154

Kasai K, Nakamura Y, White R (1990) Amplification of a variable number of tandem repeats (VNTR) locus (pMCT 118) by the polymerase chain reaction (PCR) and its application to forensic science. J Forensic Sci 35:1196–1200

Krüger J, Fuhrmann W, Lichte KH, Steffens C (1968) Zur Verwendung des Polymorphismus der sauren Erythrocytenphosphatase bei der Vaterschaftsbegutachtung. Dtsch Z Gerichtl Med 64: 127–146

Lander ES (1989) DNA fingerprinting on trial. Nature 339:501–505

Lander ES (1991) Invited editorial: Research on DNA typing catching up with courtroom application. Am J Hum Genet 48:819–823

Lewontin RC, Hartl LH (1991) Population genetics in forensic DNA typing. Science 254:1745–1750

Nakamura Y, Leppert M, O'Connell P, Roger W, Holm T, Culver M, Martin C, Fujimoto E, Hoff M, Kulmin E, White R (1987) Variable number of tandem repeat (VNTR) markers for human gene mapping. Science 235:1616–1621

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

Skowasch K, Schürenkamp M, Rand S (1992) Comparison of population data using 4 AMPFLP systems. In: Rittner C, Schneider P (eds) Advances in Forensic Haemogenetics 4. Springer, Berlin Heidelberg NewYork, pp 75–77

Wong Z, Wilson V, Patel I, Povey S, Jeffreys AJ (1987) Characterisation of a panel of highly variable minisatellites cloned from human DNA. Am J Hum Genet 51:269–288