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PCR Amplification of Alleles at the DIS80 Locus: Comparison of a Finnish and a North American Caucasian Population Sample, and Forensic Casework Evaluation

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Summary

Allele and genotype frequencies for the highly polymorphic D1S80 locus were determined in a Finnish population sample by using PCR followed by high-resolution PAGE and silver staining, a procedure called the amplified-fragment-length polymorphism (Amp-FLP) technique. In 140 unrelated Finnish individuals 15 alleles and 43 phenotypes were observed. The D1S80 locus demonstrated a heterozygosity of .77, and the power of discrimination was .92 in this sample representing a genetically isolated Finnish population. The distribution of observed genotypes conformed to Hardy-Weinberg expectations. In 36 mother-child pairs Mendelian inheritance for the alleles at the D1S80 locus could be demonstrated in all cases, and no mutations were observed. The usefulness of the D1S80 locus for forensic casework was assessed by using Amp-FLP analysis of the D1S80 locus in 36 forensic cases including 18 rapes, 14 homicides, and 4 other violent crimes. In most cases valuable information was obtained using the Amp-FLP technique, and in no case was there indication of either false-positive or false-negative results.

Introduction

Recent studies on the human genome have revealed an increasing number of DNA segments that display a high degree of polymorphism. Some of the most polymorphic loci contain VNTR "core" units consisting of noncoding sequences of nucleotides (Nakamura et al. 1987). These VNTR loci tend to be very discriminatory and suitable for genetic characterization of individuals. RFLP analysis using bacterial restriction enzymes and Southern hybridization (Southern 1975) has been a common method of identifying these DNA polymorphisms.

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The RFLP analysis, however, is time consuming and requires the use of radioactive isotopes to detect small quantities of DNA as well as intact high-molecular-weight DNA (Jeffreys et al. 1985; Wong et al. 1987). In forensic casework the latter criterion may not be met at times. Instead, limited amounts of biological material and/or degraded low-molecular-weight DNA can be anticipated for many forensic case samples. A recent development in DNA technology, the use of PCR for amplifying DNA sequences (Saiki et al. 1985; Mullis and Faloona 1987), provides a valuable tool for overcoming some of these limitations. By means of appropriate DNA primers, which are complementary to sequences flanking the repeat region of the VNTR locus, it is possible to amplify the target sequence, with the aid of the *Thermus aquaticus* (*Taq*) DNA polymerase. After amplification of the repetitive core sequences the amplified DNA can be resolved using high-resolution PAGE. The different-sized DNA fragments representing alleles can then be visualized

using an inexpensive silver-staining procedure (Allen et al. 1989). This kind of analysis, the amplified-fragment-length polymorphism (Amp-FLP) technique (Budowle et al. 1991; Sajantila et al. 1991*b*), has been shown to be suitable for the analysis of highly polymorphic VNTR regions such as the hypervariable region close to the 3′ end of the apolipoprotein B gene in chromosome 2 (Boerwinkle et al. 1989; Ludwig et al. 1989), the D17S30 locus in chromosome 17 (Horn et al. 1989) (also designated D17S5; Odelberg et al. 1989), and the D1S80 locus in chromosome 1 (Kasai et al. 1990; Budowle et al. 1991). They can be used for human identification in cases of homicide (Vuorio et al. 1990) and sexual assault (von Beroldingen et al. 1989), for identification of unknown human remains (Hochmeister et al. 1991; Sajantila et al. 1991*b*), or for paternity testing (Helminen et al., in press).

The present paper presents (*a*) data supporting the usefulness that Amp-FLP analysis of the D1S80 locus has for genetic characterization of biological materials and (*b*) the potential application of this approach to forensic analyses. In addition, allele frequencies in a genetically isolated Finnish population are presented and compared with a more heterogeneous U.S. Caucasian population sample.

Material and Methods

DNA Samples

For allele and genotype frequency determinations DNA was extracted from blood (drawn in EDTA tubes) from 140 unrelated Finnish individuals (from 68 random volunteers and from unrelated persons in 36 paternity cases). To assess the applicability of Amp-FLP analysis of D1S80 locus in forensic casework 36 forensic cases were analyzed at the National Public Health Institute, Laboratory for Forensic Serology, Helsinki. All of the cases included both a blood sample from the victim and one or more evidence samples. Some of the cases also included a blood sample from one or more suspects.

DNA Extraction from Blood and Blood Stains

DNA was isolated using the chelex resin-extraction approach as described by Singer-Sam et al. (1989) and Walsh et al. (1991). Whole blood (3–5 μl) or approximately a 3-mm^2 portion of a blood stain left on a variety of evidential sample (including clothing [composed of different types of material], wood, paper, human skin, rubber, leather, and steel) was added to 1 ml of sterile H_2O and was incubated at room temperature for 15–30 min. The tubes were then centrifuged for 2–3 min at 13,000 *g* in a microcentrifuge (Heraeus Sepatech Biofuge A). All but 20–30 μl of the supernatant was removed and discarded; the remaining 20–30 μl (pellet) was then used as a source of DNA. To extract DNA from stains, the fabric was left in the tube with the pellet. A total of 170–180 μl of 5% (w/v) Chelex 100 (100–200 mesh, sodium form, biotechnology grade; Bio-Rad) was added to a final volume of 200 μl. In addition, 2 μl of proteinase-K (10 mg/ml) was added, and the samples were incubated for 15–30 min at 56°C. After incubation the samples were vortexed at high speed for 5–10 s and were incubated in boiling water for 8 min. Then the samples were vortexed for 5–10 s and were centrifuged at 13,000 *g* for 2–3 min. A 10–20 μl aliquot of the supernatant was taken for the PCR. The remainder of the supernatant was stored frozen.

DNA Extraction from Postcoital Samples

Vaginal swabs were incubated in 1 ml of sterile H_2O at room temperature for 30 min and then were stirred with a sterile toothpick for 2 min to agitate the cells off the substrate. The swab and toothpick were then removed. The sample was spun in a microcentrifuge for 1 min at 13,000 *g*, and all but 50 μl of the supernatant was used for radioimmunoassay (RIA) of human prostate-specific acid phosphatase for the detection of human semen in the specimens (Vihko et al. 1981). The pellet was resuspended in the remaining 50 μl, and H_2O was added to final volume of 200 μl to be used for differential extraction (Gill et al. 1985) as follows: After addition of 2 μl of proteinase-K (10 mg/ml), the sample was incubated at 56°C for 1 h. The sample was then centrifuged in a microcentrifuge for 5 min at 13,000 *g*. A total of 150 μl of the supernatant (e.g., female fraction) were removed for epithelial cell DNA analysis, and the remaining 50 μl (e.g., male fraction) were resuspended and washed in 0.5 ml of buffer containing 10 mM Tris-HCl pH 7.5, 10 mM EDTA, 50 mM NaCl, and 2% SDS. The male fraction of the sample was then vortexed and was centrifuged for 5 min at 13,000 *g*, and all but 50 μl of the supernatant was discarded. Washing was repeated two times and was followed by one wash with 1 ml of distilled H_2O, and again all but 50 μl of the supernatant was discarded. A total of 150 μl of 5% (w/v) chelex 100 (100–200 mesh, sodium form, biotechnology grade; Bio-Rad), 2 μl of proteinase-K (10 mg/ml), and 7 μl

of 1 M DTT was added to the resuspended sample. Afterward, the sample was incubated at 56°C for 30–60 min, was vortexed for 5–10 s, was centrifuged at 13,000 *g* for 10–20 s, and was boiled for 8 min. A total of 50 μl of 20% (w/v) chelex was added to the 150-μl female fraction removed for epithelial cell DNA analysis. This female fraction of the sample was vortexed for 5–10 s, was centrifuged at 13,000 *g* for 10–20 s, and was boiled for 8 min. After boiling, both samples were vortexed at high speed for 5–10 s and then were centrifuged in a microcentrifuge for 2–3 min at 13,000 *g*. From each fraction 1–20 μl of supernatant was taken for the PCR reaction.

DNA Amplification

DNA amplification was performed using the primers described by Kasai et al. (1990). The D1S80 locus-specific primers were a 28-mer (5′-GAAACTGGCCTCCAAACACTGCCCGCCG-3′) and a 29-mer (5′-GTCTTGTTGGAGATGCACGTGCCCCTTGC-3′). Each PCR mixture contained 1–20 μl of chelex-extracted DNA, 50 mM Tris-Cl pH 8.8, 15 mM $(NH_4)_2SO_4$, 1.5 mM $MgCl_2$, 0.01% gelatin, 0.1% Triton-X 100, 1.0 μM of each primer, 200 μM of each dNTP, and 2.5 units of *Taq* DNA polymerase (Promega). The total volume of each sample was brought up to 50 μl or 100 μl. Before PCR each sample was overlaid with two drops of mineral oil. A total of 25 cycles were carried out for all samples. Each cycle consisted of denaturation at 95°C for 1 min, primer annealing at 65°C for 1 min, and primer extension at 70°C for 8 min. Prior to the first cycle a "hot start" was performed as follows: first a mixture containing the DNA sample and all PCR reagents except the *Taq* DNA polymerase was heated at 95°C for 5 min and then the temperature was decreased to 80°C and *Taq* DNA polymerase was added (D'Aquila et al. 1991).

Gel Electrophoresis of Amplified DNA

To resolve the amplified DNA fragments gel electrophoresis was performed using vertical, 750-μm-thick polyacrylamide gels. The gels consisted of upper (stacking) and lower (resolving) gels. The 4-cm stacking gel was 3% T, 1.6% C, 7% glycerol, and 33 mM Tris-sulfate pH 9.0, and the 12-cm resolving gel consisted of 6% T, 1.6% C, 7% glycerol, and 33 mM Tris-sulfate pH 9.0. A total of 10 μl of the PCR products and 2 μl of loading buffer (20% sucrose, 12.5% ficoll, and 0.2% bromophenol blue) were loaded to each lane. Electrophoresis was carried out in an LKB 2001 vertical electrophoresis unit using 90 mM Tris, 90 mM boric acid, and 2 mM EDTA (TBE) buffer pH 9.0 at constant voltage (200 V) for 2 h 45 min. Alternatively, the analysis of PCR products was performed using rehydratable ultrathin-layer (5% T, 3% C, 400 μm thick) polyacrylamide gels according to a method described elsewhere (Budowle et al. 1991; Sajantila et al. 1991*b*).

Allele Marker

To simplify the determination of alleles and pheno/genotypes an allele marker representing a set of known alleles at this locus was applied to every fifth lane on the gel electrophoresis, to serve as an allele reference marker. The allele marker was originally composed of amplified DNA from subjects with known alleles and subsequently has been reamplified to serve as a reproducible allele marker (Sajantila et al. 1992).

Silver Staining

After electrophoresis the separated alleles were detected using silver staining (Allen et al. 1989; Budowle et al. 1991). For 750-μm-thick gels silver staining was performed as follows: the gel was (1) immersed in 10% ethanol for 10 min, (2) oxidized in 1% nitric acid solution for 6 min, (3) rinsed twice in distilled H_2O, (4) silver stained in 0.012 M silver nitrate solution for 30 min, (5) rinsed briefly in distilled H_2O, and (6) reduced in a solution containing 0.28 M anhydrous sodium carbonate and 0.019% formalin and then, when the desired image intensity had developed, (7) reduction was stopped with 10% glacial acetic acid, and (8) the gel was dried in a gel dryer (Bio-Rad gel dryer model 583) for a permanent record. The silver-staining process of the ultrathin-layer gels was similar, except that (*a*) processing times were shorter and (*b*) the gels were air-dried for a permanent record (Budowle et al. 1991).

Statistical Evaluations

A χ^2 test comparing observed and expected phenotypes was performed to assess whether the Finnish population sample conforms to Hardy-Weinberg equilibrium expectations. The expected genotypes were calculated, on the basis of Hardy-Weinberg equilibrium, from the obtained allele frequencies. The Finnish population sample was also compared with the U.S. Caucasian population sample by using the χ^2 test and *G*-statistics, with a program provided by G. Carmody (Carleton University, Ottawa). Statistical evaluations for the power of discrimination (PD) and

allelic diversity (h) were calculated according to methods described elsewhere (Nei and Roychoudhury 1974; Sajantila et al. 1991*a*).

Results

Allele and Genotype Analysis

After separation of the PCR products by PAGE, silver staining revealed discrete bands representing individual alleles. The bands representing allelic products ranged in size from approximately 430 bp to 750 bp. The different fragments observed represented alleles containing different numbers of the 16-bp repeat unit present at the D1S80 locus. In accordance with the protocol of Budowle et al. (1991) the smallest-sized allele observed in our analyses was tentatively designated as allele 1 and each allele containing an additional repeat was designated as 2, 3, 4, etc.

Allele 1 corresponds approximately to 430 bp when compared with a molecular-weight marker (1-kb and 123-bp ladder; BRL, Gaithersburg, MD). The 430-bp approximate size of allele 1 was independently determined by subsequent analysis using fluorescent-labeled oligonucleotide primers and internal size standards (Gene Scanner™; Applied Biosystems; Robertson et al. 1991). Therefore, allele 1 contains 18 repeats of the core unit in the D1S80 locus, and the additional repeats consist of 19, 20, 21, etc. repeats, respectively. The number of repeats for alleles at the D1S80 locus is consistent with sequencing data from Cetus (Reynolds 1991). Accordingly, a new nomenclature for the alleles was based on the number of the repetitive core sequences.

The distribution of D1S80 alleles in the Finnish population sample (representing 280 chromosomes) was determined from silver-stained gels and is shown in table 1. As noted, a total of 15 alleles were observed, and, as designated by the new nomenclature, alleles 18 (frequency .307) and 24 (frequency .311) were found to be the most common alleles. The other alleles occurred at frequencies of .007–.079, with the least frequently observed allele being allele 37.

A total of 43 different genotypes were represented in this population sample. As expected from the allele frequencies, the most common genotypes observed were the combinations of alleles 18 and 24, e.g., 18-18 (frequency .086), 18-24 (frequency .207), and 24-24 (frequency .107). Also, genotypes 18-28, 18-31, and 24-31 were observed relatively frequently (frequencies .064, .064, and .057, respectively). The observed genotypes were in agreement with the expected genotypes and conformed to Hardy-Weinberg equilibrium expectations ($.100 < P < .250$). The distributions of observed and expected genotypes are shown in table 2.

Table 1

D1S80 Allele Frequencies in Unrelated Finns and U.S. Caucasians

	Frequency in	
Allele[a] (no. of core units)	Unrelated Finns (n = 140)	U.S. Caucasians (n = 94)
1 (18)	.307	.293
2 (19)	.011	.011
3 (20)	.032	.021
4 (21)	.018	.032
5 (22)	.014	.043
6 (23)	.014	.016
7 (24)	.311	.335
8 (25)	.075	.037
9 (26)	.011	.016
10 (27)	.007	.000
11 (28)	.068	.059
12 (29)	.032	.059
13 (30)	.043	.016
14 (31)	.079	.043
20 (37)	.007	.000

[a] Nomenclature is that of Budowle et al. (1991).

An unbiased test estimate of the expected heterozygosity was calculated as h. According to the formula derived from Nei and Roychoudhury (1974), the expected heterozygosity in the Finnish population sample was .79, while the observed heterozygosity was .77. When the highly polymorphic D1S80 locus was used, PD was .92 in our population sample. In addition, a two-way R × C contingency table to determine population-sample homogeneity was used as a statistical test for significance. The data (table 1) for the D1S80 locus suggest that the Finnish population sample is similar to a U.S. Caucasian population sample ($P = .1860 \pm .0123$ in χ^2 test; $P = .2180 \pm .0131$ in G-statistic).

Family Analysis

To investigate both mutation frequency and whether inheritance at this locus conforms to Mendelian law, we analyzed 36 mother-child pairs. In all cases a maternal allele was found in the child's phenotype. In this study there was no evidence of any mutations at the D1S80 locus. In 33 of 36 cases of disputed paternity that were analyzed, an allele from the putative father was found in the child's genotype, and in the 3 cases

Table 2

Observed and Expected D1S80 Locus Genotypes in 140 Unrelated Finns

Genotype[a]	No./Frequency	
	Observed	Expected
1-1 (18-18)	12/.086	13.2/.094
1-3 (18-20)	1/.007	2.7/.020
1-5 (18-22)	1/.007	1.2/.009
1-6 (18-23)	1/.007	1.2/.009
1-7 (18-24)	29/.207	26.7/.191
1-8 (18-25)	6/.043	6.4/.046
1-11 (18-28)	9/.064	5.8/.042
1-12 (18-29)	3/.021	2.7/.020
1-13 (18-30)	2/.014	3.7/.026
1-14 (18-31)	9/.064	6.8/.049
2-7 (19-24)	1/.007	1.0/.007
2-11 (19-28)	1/.007	.2/.001
2-13 (19-30)	1/.007	.1/.001
3-7 (20-24)	1/.007	2.8/.020
3-8 (20-25)	1/.007	.7/.005
3-11 (20-28)	1/.007	.6/.004
3-12 (20-29)	2/.014	.3/.002
3-13 (20-30)	1/.007	.4/.003
3-14 (20-31)	1/.007	.7/.005
4-7 (21-24)	3/.021	1.6/.011
4-8 (21-25)	1/.007	.4/.003
4-12 (21-29)	1/.007	.2/.001
5-11 (22-28)	1/.007	.3/.002
5-12 (22-29)	1/.007	.1/.001
5-13 (22-30)	1/.007	.2/.001
6-7 (23-24)	1/.007	1.2/.009
6-8 (23-25)	1/.007	.3/.002
7-7 (24-24)	15/.107	13.5/.096
7-8 (24.25)	2/.014	6.5/.047
7-9 (24-26)	1/.007	1.0/.007
7-11 (24-28)	3/.021	5.9/.042
7-12 (24-29)	1/.007	2.8/.002
7-13 (24-30)	4/.028	3.7/.003
7-14 24-31)	8/.057	8.5/.061
7-20 (24-37)	2/.014	.6/.004
8-8 (25-25)	2/.014	.8/.006
8-11 (25-28)	2/.014	1.4/.010
8-12 (25-29)	1/.007	.7/.005
8-13 (25-30)	1/.007	.9/.006
9-9 (26-26)	1/.007	.1/.001
10-10 (27-27)	1/.007	.1/.001
13-13 (30-30)	1/.007	.3/.002
14-14 (31-31)	2/.014	.9/.006

[a] Nomenclature is that of Budowle et al. (1991) (nomenclature based on number of core units is shown in parentheses).

of exclusion a paternal allele was absent. The data were consistent with traditional paternity tests based on protein polymorphism and RFLP DNA analysis (see fig. 1).

Forensic Casework Analyses

A total of 36 forensic cases were analyzed in this study. The cases included 18 rape cases, 14 homicides, and 4 other violent attacks. Altogether, 51 semen samples (32 vaginal swabs, 17 semen stains, and 2 condoms), 45 bloodstains, 2 cigarette butts, and 63 whole-blood samples were analyzed. Amp-FLP analysis was successful on all DNA samples derived from the 63 fresh whole-blood samples.

Sexual assaults. — In 45 (88.2%) of the 51 semen samples (28 of 32 vaginal swabs, 15 of 17 semen stains, and in both of the condoms) the Amp-FLP analysis provided unequivocal results. RIA of human prostate-specific acid phosphatase for the detection of seminal fluid was positive in all except one of the 32 vaginal swabs. In 10 of the samples which gave a positive RIA result (e.g., presence of human semen was noted), no DNA profile was obtained in 4 of the samples, and in 6 samples we detected only bands consistent with the victim's DNA profile. In one sample having a negative RIA result, we observed in the male fraction a DNA pattern that could not have originated from the victim. The suspect's blood sample was not available in this case.

The victim's phenotype was detected in 14 (43.8%) female fractions and in 11 (34.5%) male fractions after differential extraction of 32 vaginal swabs. Either the suspect's or an unknown profile was observed in 17 (53.1%) male fractions but never was observed in a female fraction. It is interesting that a complex DNA profile was observed in two samples of mixed origin after differential extraction. In the first sample, the female fraction contained five bands, and the male fraction revealed three bands; in the other sample the male fraction revealed both the victim's and the suspect's DNA profile. The difference in the intensity of the amplified DNA fragments was such that it was easy to determine the contributors of each band in all these cases. No DNA profile was detected in 18 (56.3%) female fractions and in 4 (12.5%) male fractions. The number of cases, number of samples, and success rate in Amp-FLP analysis are given in table 3. In conclusion, DNA profiles that did not match the victim were detected in 11 (61.1%) of the 18 rape cases examined in this study.

Homicides and other crimes. — The PCR products could be analyzed in 31 (72.1%) of the 43 bloodstain samples. Both of the cigarette butts could be analyzed with Amp-FLP technique. The number of cases, number of samples, and success rate in Amp-FLP analysis from

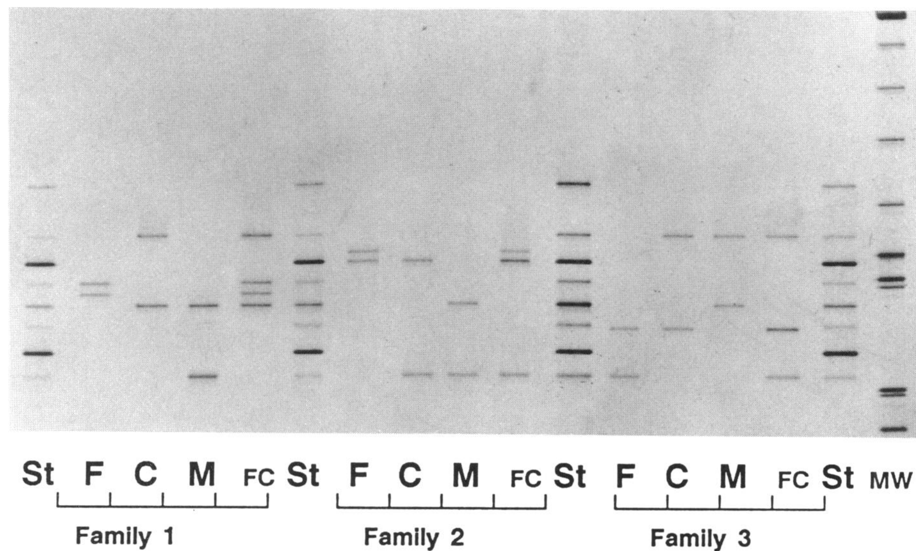

Figure 1 Paternity test performed using Amp-FLP analysis of D1S80 locus. St = standard composed of amplified DNA from subjects with known alleles (alleles are designated 18, 20, 22, 24, 26, 28, 31, and 37 [from bottom to top], as described in the text); F = alleged father; C = child; M = mother; FC = alleged father and child run together on the same lane; MW = molecular-weight marker composed of 1-kb and 123-bp ladder (BRL, Gaithersburg, MD).

bloodstains obtained from different material are shown in table 3 (also see fig. 2).

Discussion

In vitro amplification of DNA via PCR is one of the technical achievements of molecular biology that will have a profound impact on forensic science. The first reports of PCR performed on forensic samples were presented by Bugawan et al. (1988) and Higuchi et al. (1988). Since PCR has some indisputable advantages over the existing serological and RFLP DNA methodology used at present in forensic casework, potential applications of PCR are being investigated intensively.

One approach to analyze PCR products in forensic work is dot-blot hybridization with allele-specific oligonucleotide (ASO) probes. An example of this kind of approach is the amplification of the human leukocyte antigen (HLA) DQα locus (Reynolds and Sensabaugh 1991; Comey and Budowle 1991). In this region six different alleles or 21 genotypes can be distinguished by using a commercially available kit (AmpliType™ HLA-DQα forensic DNA amplification and typing kit; Cetus). We have determined the allele and genotype frequencies of HLA-DQα gene in the Finnish population and have concluded that the Finnish allele frequencies do not differ from those determined in other Caucasian populations (Sajantila et al. 1991*a*).

As a second approach for applying PCR in forensic analysis the Amp-FLP technique has become increasingly studied. In particular, the analysis of the D1S80 (MCT118) locus by using the Amp-FLP technique has been studied previously for population genetics (Kasai et al. 1990; Budowle et al. 1991), identification of extensively burned fire victims (Sajantila et al. 1991*b*), and identification of human remains (Hochmeister et al. 1991*a*). Budowle et al. (1991) reported 16 alleles in a population sample consisting of 99 unrelated U.S. Caucasians. They also observed a Mendelian inheritance in two families (10 individuals in 2 generations and 18 individuals in 4 generations). Kasai et al. (1990) detected 21 alleles in 67 unrelated Caucasian and Japanese individuals and observed a Mendelian pattern of inheritance in 16 individuals in Japanese families.

In our study, 15 different alleles were identified, demonstrating that the D1S80 locus is highly polymorphic also in the genetically isolated Finnish population. In our family study a Mendelian pattern of inheritance was observed in 36 different Finnish mother-child pairs. In 33 cases where the child shared a band with the alleged father the paternity also was confirmed with traditional genetic markers as well as

Table 3

Amp-FLP Analysis of 36 Forensic Casework Samples

Cases and Source of Samples (no. of cases)	No. of Samples	Success Rate
Sexual assault (18):		
Vaginal swabs	32	28 (87.5%)
Semen stains	17	15 (88.2%)
Condoms	2	2 (100.0%)
Total	51	45 (88.2%)
Homicides and violent attack (18):		
Bloodstains from		
Clothing	21	16 (76.2%)
Wood	3	3 (100.0%)
Paper	1	1 (100.0%)
Human skin	1	1 (100.0%)
Rubber	2	1 (50.0%)
Leather	5	4 (80.0%)
Steel (knives)	10	5 (50.0%)
Subtotal	43	31 (72.1%)
Cigarette butts (saliva)	2	2 (100.0%)
Total	45	33 (73.3%)

with RFLP hybridization analysis using single-locus VNTR probes. Accordingly, in the three cases where paternity was excluded by Amp-FLP analysis at the D1S80 locus, the result was consistent with the findings obtained using traditional paternity tests and RFLP analysis (data not shown). Furthermore, our population sample appears to meet Hardy-Weinberg expectations for the D1S80 locus, and the allele frequencies observed are similar to those determined in a U.S. Caucasian population sample. Although only 43 of the 120 potential genotypes were observed in this study, the occurrence of the so far undetected

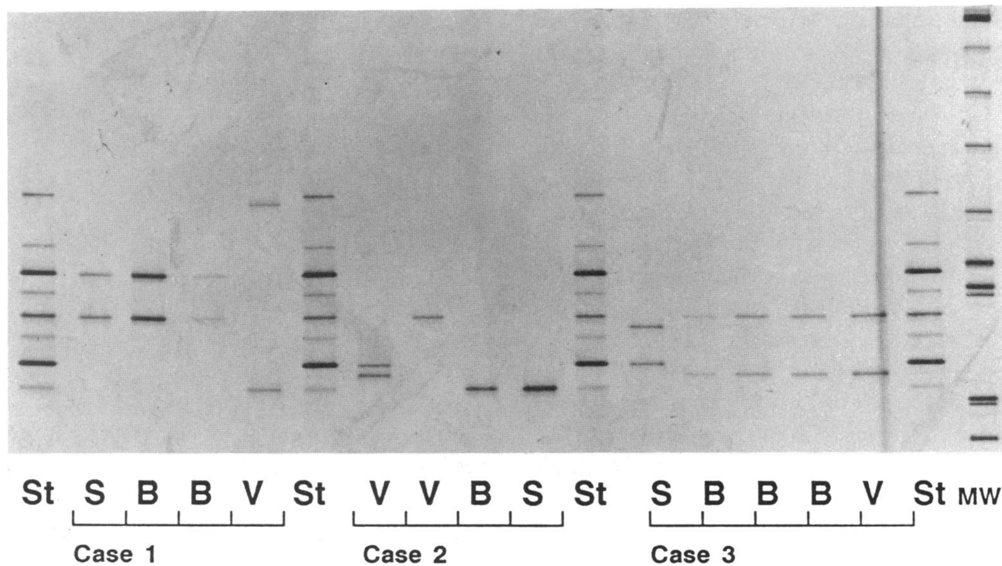

Figure 2 Amp-FLP analysis of D1S80 locus in three different forensic cases. S = suspect; B = bloodstain; and V = victim. Other lane designations are as in fig. 1. It should be noted that in case 2 there were two different victims and that in case 3 three different bloodstains (B) was extracted and analyzed.

genotypes can thus be predicted on the basis of the assumption of Hardy-Weinberg equilibrium.

The nomenclature for the alleles at the D1S80 locus has not been standardized. We have followed both the allele designation suggested by Budowle et al. (1991), and our new nomenclature. In the nomenclature tentatively suggested by Budowle et al. (1991), the smallest-sized allele was designated as allele 1 and the subsequent larger alleles were designated as 2, 3, etc. The limitation of this approach is that alleles smaller than allele 1 have to be given a negative designation after subsequent detection, a procedure which may have to be repeated as new rare alleles are identified. The designation of alleles should be standardized, and we now suggest that the logical designation should be based on the number of the repeated "core" units contained within a DNA fragment; that is, an allele containing 25 repeat units would be designated as allele 25. Allele size analysis using the Gene Scanner (Applied Biosystems, Foster City, CA) has indicated that allele 1 corresponds to 18 repetitive core units at the D1S80 locus. Therefore, the larger alleles based on additional 16-bp core units can be designated 19, 20, 21, etc. This type of approach is similar to those suggested by Kasai et al. (1990) and Boerwinkle et al. (1989), regarding the nomenclature of the D1S80 locus and the hypervariable region adjacent to the 3′ end of the apolipoprotein B gene, respectively.

Although the D1S80 locus has a high (.92) discrimination potential even in the genetically isolated Finnish population, there will be times when the significance or weight of a match will not be as informative as desired. For example, alleles 18 and 24 account for 61.8% of all observed chromosomes. Therefore the significance of a match with genotypes 18-18 (frequency .086), 18-24 (frequency .207), and 24-24 (frequency .107) will be less than that noted for other genotypes. A similar predominance of alleles 18 and 24 (designated as alleles 1 and 7) was also detected in a U.S. Caucasian population sample (Budowle et al. 1991). In contrast, the remaining observed D1S80 genotypes have frequencies of only .007–.064 in the Finnish population, which affords a high degree of discrimination. In order to obtain a high degree of individualization by using Amp-FLP technique, additional genetic markers may be utilized for the characterization of evidentiary material.

Our study demonstrates that Amp-FLP analysis of the D1S80 locus can be applied to routine forensic casework that provides DNA extracted from vaginal swabs, semen stains, bloodstains, condoms, and even cigarette butts. Hochmeister et al. (1991*b*) have previously studied both the extraction of DNA and Amp-FLP analysis of the D1S80 locus from cigarette butts. They successfully typed 99 of 100 cigarette butts for both HLA-DQα and D1S80 loci by using PCR and subsequent dot-blot and Amp-FLP analysis, respectively. In our study we examined two cigarette butts, and they both were successfully typed for the D1S80 locus by using the Amp-FLP technique. PCR-based analyses of human remains (Hochmeister et al. 1991*a*) and of fire victims (Sajantila et al. 1991*b*) also have been reported. But, to the best of our knowledge, there are no reports on D1S80 Amp-FLP analysis of DNA recovered from vaginal swabs, semen stains, and bloodstains from different material. We could successfully type 88.2% of the semen samples and 72.1% of the bloodstain samples. This demonstrates that Amp-FLP typing of the D1S80 locus from DNA from a wide variety of forensic specimens can be performed successfully.

von Beroldingen et al. (1989) previously reported the use of differential extraction for PCR-based analyses in forensic casework. They applied differential extraction to a case where the victim had sexual intercourse with her husband 9 h before the alleged sexual assault, and they used ASO hybridization in a dot-blot format for detecting HLA-DQα genotypes. Our study confirms the applicability of differential extraction for PCR-based analysis of vaginal swabs. In some of our differential experiments the sperm fraction seemed to include, in addition to the sperm cells, female cells in approximately 12.4% of the samples. This resulted in a DNA profile of more than two bands on the silver-stained gels, but generally this did not confound the interpretation of the results. It is actually possible that these female bands in either the male fraction or the lysed-cell female fraction could serve as internal quality controls.

To conclude, we have provided additional evidence for the applicability of Amp-FLP analysis of the highly polymorphic D1S80 locus in forensic casework. The technique is relatively simple, fast, and nonlaborious and does not require the use of radioisotopes. In combination with the D1S80 locus, other VNTR regions—such as the D17S30 locus and the hypervariable regions close to the 3′ end of the apolipoprotein B gene and the col2A1 gene—should provide a powerful

new forensic tool for the DNA characterization of identity.

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