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Amplification of VNTR Locus D1S80 in DNA Recovered from Vaginal Sperm

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=Abstract=**Sperm DNA was isolated from vaginal fluid and/or endocervical mucoid plugs at various postcoital intervals. Samples were obtained from 25 volunteer couples. DNA was also prepared from the blood and semen of the couples. The DNA was amplified by polymerase chain reaction (PCR) with D1S80 primers. The Fragment length polymorphism of the amplified products (AMP-FLP) was analysed by acrylamide gel electrophoresis followed by ethidium bromide staining. The AMP-FLP of the vaginal fluid was identical to that of the blood of the male partner within 10 hours after coitus. Thereafter, the male DNA recovery rate reduced as the collection interval extended. After 76 hours no male DNA profile was detected in the vaginal sample. Therefore, AMP-FLP analysis of the D1S80 can be used to exclude to determine the probable identity of an assailant in rape cases.**

Key Words: *PCR, VNTR, D1S80, Sperm, Vaginal fluid*

INTRODUCTION

Phenotype makeup determined in biological materials recovered as criminal evidence has been used as a potent tool of personal identification or exclusion. In cases of sexual assault, examination of spermatozoa or prostatic acid phosphatase has been performed in order to indicate whether the sexual activity has occurred (Dahlke *et al.* 1977; Schumann *et al.* 1976; Soules *et al.* 1978), but the identity of the assailant can not be determined with these results. Comparison of pubic hairs retrieved from the victim and a particular suspect has been used (Soules *et al.* 1978), but the information may be so non-specific and not so exclusive as to limit its usefulness as an identity test. Analysis of protein polymorphism in semen recovered from the vagina or stains has been tried. The ABO blood group (Newall 1981) and HLA antigens (Fowler *et al.* 1985; Regueiro *et al.* 1984) have been typed on sperm, but the limited polymorphic diversity of the ABO blood group and degradation of the proteins because of the limited amount obtained in criminal specimens have provided difficulties in their practical use.

An important development in the field of forensic science has been the use of DNA typing. The human genome contains large amounts of repetitive DNA sequences, some of which are arranged as tandem repeats. Tandem repeat units are termed Variable Number of Tandem Repeats (VNTRs). The number of repeat units present is so different among individuals in some genetic loci as to enable individual discrimination by fragment length polymorphism analysis of the VNTR loci. Identity tests using resriction fragment length polymorphism (RFLP) have been applied in the fields of parenthood testing(Baird *et al.* 1986; Honma *et al.* 1987; Jeffreys *et al.* 1985) and forensics (Gill *et al.* 1985

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and 1987; Giusti *et al.* 1986; Honma *et al.* 1989; Kanter *et al.*1986). Although extremely effective for VNTR analyses, the RFLP method using Southern blotting is time consuming and requires a radioisotopic assay to achieve the appropriate sensitivity to detect VNTR alleles in human samples containing a limited amount of DNA (Budowle and Baechtel 1990)

PCR (Saiki *et al.* 1985) can substitute for RFLP analysis in some VNTR loci, particularly when only limited quantities of DNA are available. The use of PCR can eliminate the need for isotopic assay and reduce time and cost. Thus, amplification of the appropriate VNTR loci by PCR could be a useful tool for identity testing. In fact, using PCR followed by electrophoretic separation of the amplified fragments (PCR-FLP), the D17S30 locus (Horn *et al.* 1989), the 3' hypervariable region of the apolipoprotein B gene (Boerwinkle *et al.* 1989; Ludwig *et al.* 1989) and the D1S80 locus (Budowle *et al.* 1991; Kasai *et al.* 1990; Kasai and Mukoyama 1990) have been analysed.

The current report describes PCR amplification of DNA recovered from the vagina and endocervical mucoid plug in relation to variable postcoital intervals. Male-specific DNA is purified from these samples, and DNA is also isolated from the blood of the male sexual partner. Both DNAs are amplified by the PCR technique followed by electrophoresis for polymorphic size comparison. This would provide valuable information in identifying a rape assailant.

MATERIALS AND METHODS

Vaginal fluid and/or endocervical mucoid plug were obtained from the female sexual partners of 24 volunteer couples at variable postcoital intervals as illustrated in Table 1. Vaginal fornix and uterine cervix were irrigated with 1 ml of physiological saline and fluid was collected in the fornix. The endocervical mucoid plug was gently sucked with a flexible 18 gage needle. Samples were left untreated and stored at 4℃ until processed. Blood was collected from the couples in Vacutainer tubes containing potassium ethylenediaminetetraacetate (EDTA) for comparison studies as well as semen samples from the male partners.

DNA was isolated from peripheral blood as described by Kunkel *et al.* (1982). Sperm DNA was purified from vaginal and endocervical samples as described by Giusti *et al.* (1986) with some modifications. The sample was first suspended in 10 ml of PBS-2% Sarkosyl, mixed briefly and centrifuged at 3500 rpm at 4℃. This process was repeated two more times, for a total of three washes and centrifugations. The pellet was then resuspended in 2 ml of PBS, and proteinase K and sodium dodecyl sulfate (SDS) were added to final concentrations of 100 ug/ml and 1%, respectively. After overnight incubation at 37℃ with mild agitation, samples were washed twice with PBS-2% Sarkosyl, and examined under the light microscope to analyse the complete lysis of female cells. If female cells were present, redigestion with proteinase K and SDS continued until the female cells disappeared entirely. Sperm heads were pelleted by centrifugation as above and resuspended in 1.5 ml of PBS-2% Sarkosyl containing 100 ug/ml proteinase K, 39 mM dithiothreitol (DTT), 1% SDS and 10 mM EDTA and incubated overnight at 37℃ on a rocking platform. Then, the DNA was purified by organic extraction and ethanol precipitation as described for blood samples.

Semen samples were washed with PBS-2% Sarkosyl twice and incubated overnight at 37℃ in 10 mM Tris-HCl, 10mM EDTA, 100 mM NaCl (pH 8.0) containing 2% SDS, 20 ug/ml proteinase K and 39 mM DTT. The DNA was purified as above.

The amount of human DNA recovered was quantified by DNA fluorometer (TKO 100 Fluorometer, Hoeffer Scientific Instruments).

Amplification of D1S80 was achieved using the primers described by Kasai *et al.* (1990). The primers were 5'-GAAACTGGCCTCCAAACACTGCCCGCCG-3' and 5'-GTCTTGTTGGAGATGCACGTGCCCCTTGC-3'. Two nanograms of each sample were amplified in 25 μl of a reaction mixture containing 67 mM Tris-HCl (pH 8.3), 6.7 mM magnesium chloride, 16.6 mM ammonium sulfate, 10 mM 2-mercaptoethanol, 170 μg/ml bovine serum albumin, 10% dimethyl sulfoxide, 2.5 mM each of the deoxy forms of adenosine triphosphate, cytidine triphosphate, guanosine triphosphate, thymidine

triphosphate, 1.25 units of Taq polymerase, and 2 μM of each primer. After denaturation of the DNA at 95℃ for 1 min, annealing was done at 65℃ for 1 min, with an extension at 70℃ for 8min, and repeated for 30 cycles. Polymorphism of the amplified products was analysed by ethidium bromide staining after electrophoresis on polyacrylamide gels.

RESULTS

A total of 119 vaginal and/or endocervical samples were collected from 25 volunteer couples at variable postcoital intervals. The female cells contaminating the sample were removed by two-step, or differential lysis method. After purification, the DNA was quantified by DNA fluorometer and DNA was detected in a total of 78 out of 119 samples. In the polymorphism analysis of the PCR-amplified products (AMP-FLPs), 74 samples exhibited the male pattern as illustrated in Fig. 1, and 4 samples

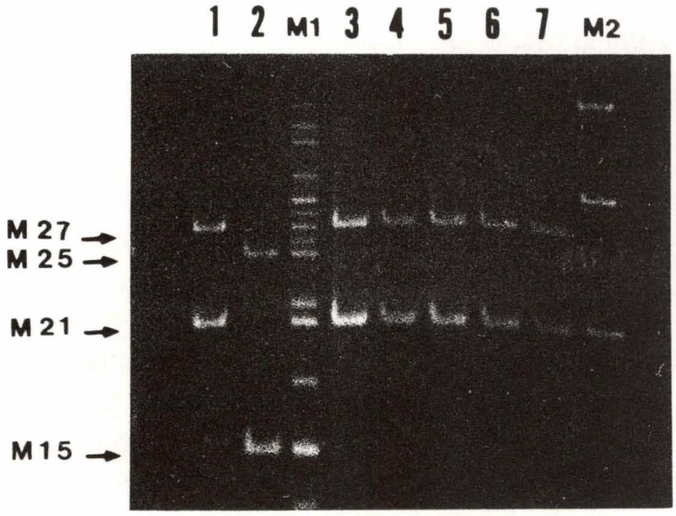

Fig. 1. Comparison of D1S80 AMP-FLPs between peripheral blood DNA and vaginal sperm DNA. AMP-FLP of vaginal sperm DNA is identical to that of male peripheral blood. Lane 1, male peripheral blood; Lane 2, female peripheral blood; Lane 3, vaginal fluid, postcoital 5 hours; Lane 4, vaginal fluid, postcoital 13 hours; Lane 5, vaginal fluid, postcoital 34 hours; Lane 6, vaginal fluid, postcoital 41 hours; Lane 6, vaginal fluid, postcoital 55 hours; Lane 7, vaginal fluid, postcoital 68 hours; M1, size marker made by author; M2, 123 bp DNA ladder.

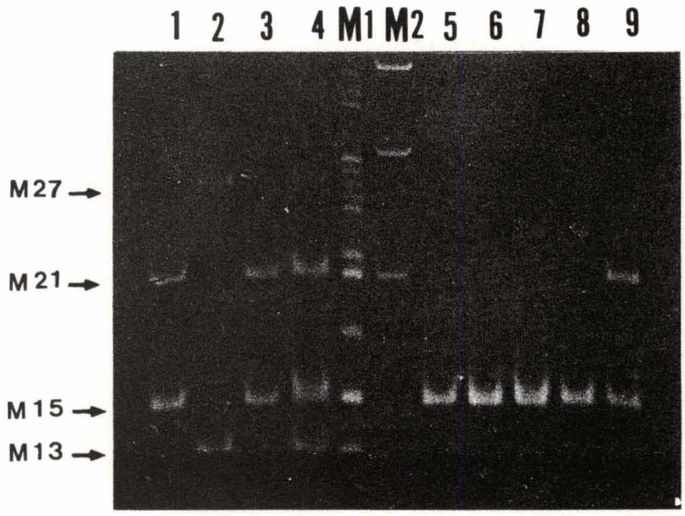

Fig. 2. Comparison of D1S80 AMP-FLPs between peripheral blood DNA and vaginal sperm DNA. Lane 1, male peripheral blood (M21, M15); Lane 2, female peripheral blood (M27, M13); Lane 3, vaginal fluid, postcoital 9 hours; Lane 4, vaginal fluid, postcoital 14 hours; Lane 5, vaginal fluid, postcoital 41 hours; Lane 6, endocervical plug, postcoital 41 hours; Lane 7, vaginal fluid, postcoital 54 hours; Lane 8, endocervical plug, postcoital 54 hours; Lane 9, vaginal fluid, postcoital 33 hours; M1, size marker made by author; M2, 123 bp DNA ladder. In lane 4, both male and female patterns are demonstrable and in lane 5-8, only low molecular fragment of male AMP-FLP (M15) is visible.

showed only the female profile. Thus, the male DNA recovery rate in the postcoital vaginal fluid using a DNA fluorometer and PCR-amplification of D1S80 was 100% at 2-10 hours after coitus, 75% at 11-15 hours, 62.5% at 16-20 hours, 94.7% at 28-35 hours, 62.5% at 36-42 hours, 60% at 52-60 hours, and 18.2% at 61-68 hours (Table 1). After 76 hours, male DNA was not detected in the vaginal fluid or endocervical mucoid plugs.

The band profile of 13 samples was not identical to that of the male partner, though quite different from that of the corresponding female, only showing the identity in the smaller fragment of the male partner as illustrated in Fig. 2. This disparity between the postcoital vaginal samples and the male profile from blood or semen was not related to the postcoital interval of sampling. Although the

Table 1. Postcoital interval of sampling and male DNA recovery rate

Postcoital Sampling Interval (hr)	No. of Samples	Samples of Male DNA Recovered & Amplified	Male DNA Recovery Rate (%)
2～ 5	9	9	100.0
6～10	12	12	100.0
11～15	12	9	75.0
16～20	16	10	62.5
28～35	19	18	94.7
36～4ㄴ	16	10	62.5
52～60	10	6	60.0
61～68	11	2	18.2
76～88	14	0	0.0
Total	119	74	

PCR amplification was repeated two to three times with various PCR conditions, the result remained unaltered.

There were one or two bands detectable in the polyacrylamide gels subsequent to ethidium bromide staining in most of the vaginal samples, which were identical to those of the male partner. Though a few samples presented one or two extra-bands different from the male profile, they were identical to that of female blood (Fig. 2)

DISCUSSION

Giusti *et al.* (1986), examined the applicability of VNTR polymorphism to the analysis of the DNA recovered from postcoital vaginal fluid, using the Southern blot analysis technique, where the actual DNA recovery was mostly confined to the samples collected within 30 min after coitus. This would be attributed to the amount of DNA isolated from the samples, because the routine Southern blot technique requires a large amount of DNA, and isolation of DNA in samples, especially in forensic samples, is limited in amount. Furthermore, with routine Southern blot analysis, discrimination of VNTR alleles may be not possible since the alleles differ by one or a few repeat units. The AMP-FLP analysis of the VNTR loci can overcome these kinds of disadvantages frequently encountered in the Southern blotting of forensic samples.

The alleles associated with the D1S80 locus are resolved into discrete entities using the AMP-FLP analytical technique (Budowle *et al.* 1991; Kasai *et al.* 1990), 21 alleles are reported in Caucasian and Japanese individuals (Kasai *et al.* 1990). In choosing the marker for identity tests, the more numerous in alleles and the more evenly distributed in the population, the more useful for identification of the individuals. In this aspect, the AMP-FLP analysis of D1S80 can be a useful and powerful tool for identity investigation, since its alleles are numerous and rather widely distributed. In the present paper 17 alleles are detected among 50 unrelated individuals, and corresponding partners can be discriminated by their polymorphism (data not shown). Practically the calculation of probability is essential in identity tests of forensic samples. Therefore, in order to apply an analysis of the D1S 80 locus to forensics, the allele frequency or phenotype frequency should be investigated in the corresponding ethnic group or population.

Male DNA profiles are present in all of the vaginal samples within 10 hours after coitus, but the male DNA recovery rate reduces in the sample collected thereafter, falling to 18.2% after 61-68 hours, and it is not detectable after 76 hours. The reduction of the male DNA recovery rate in relation to the sample collection time might be due to degradation of the sperm DNA in the vagina. Since the DNA recovery rate is 94.7% in the samples collected after 28-35 hours, quite a lot higher than that after 11-20 hours, it is suggested that the reduction within 20 hours after coitus might not be induced by degradation of the male DNA in the vagina, but that it is more probably due to some problems in DNA isolation from the samples, in this paper especially in differential lysis.

In 13 samples only the smaller allele was detectable in spite of repeated amplicifications under various PCR conditions. The efficiency of amplification or yield of PCR products is related to the length of the target site between the primers. Horn *et al.* (1989) observed that larger alleles could be amplified to a significantly less extent than smaller ones in the amplification of the D17S30 locus. In contrast, Budowle *et al.* (1991) observed no apparent

difference in band intensity between the largest and the smallest alleles of the D1S80 locus. In the current study, the larger fragments seem to be stained by ethidium bromide more intensely than the smaller ones. Since the occurrence of these findings is not related to the sample collection interval and the rest of the samples show a rather uniform band intensity, it is difficult to attribute this phenomenon to the property of the amplified products of the D1S80 locus. The author performed the AMP-FLP analysis of the D1S80 locus with degraded DNAs and compared the results to those of the corresponding intact ones, only accomplishing an amplification of the smaller fragment. Therefore, the amplification of the smaller fragment only in the 13 samples seems to be due to the degradation of DNA during isolation. Actually, male DNA isolation from vaginal samples requires more sophisticated processing steps than those of conventional DNA isolation.

The presence of the female profile in the vaginal samples does not interfere with the discrimination of the male partner in this paper. In contrast, the stringent differential lysis of the specimen for obtaining only the male DNA may induce the degradation of DNA as described, which would probably result in an incomplete or unsuccessful discrimination. It is suggested that, in the AMP-FLP analysis of the vaginal samples, the stringent procedure for male DNA isolation would not be advisable if the profile of the female and related individuals is available by another sampling source or is already known.

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=국 문 초 록=

질내정자에서 추출한 DNA에서 D1S80의 PCR 증폭

서울대학교. 의과대학 법의학교실

이 정 빈

25쌍의 부부에서 성교 후 일정한 시간간격을 두고 질액과 자국경관내 점액을 채취하여 정자 DNA를 추출하고 이들 부부의 혈액 및 정액에서 DNA를 추출하여, PCR 증폭법을 이용하여 D1S80 locus를 증폭하였다. 그 증폭산물을 polyacylamide gel에 전기영동한 후 ethidium bomide로 염색하여 절편다형상을 비교검색하였다. 그 결과 질액이나 자국경관내 점액에서 성교 후 10시간까지 남성 DNA상이 검출되었고 그 이후 검출률이 점점 떨어져 76시간 이후에는 남성 DNA상이 보이지 않았다. 위 결과를 보면 D1S80 증폭법은 강간 사건에서 범인색출에 유용하게 쓰여질 것으로 여겨진다.