APPLICATION OF DNA POLYMORPHISMS TO THE FORENSIC EXAMINATION OF SEMEN

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Abstract

We have developed a procedure for the isolation of sperm DNA from semen samples collected after sexual activity. The use of a differential lysis procedure allows the recovery of sperm free of female cells. DNA was purified from these samples, digested with particular restriction endonucleases, fractionated by agarose gel electrophoresis, and hybridized with specific recombinant DNA probes that recognize two highly polymorphic DNA sequences. DNA was purified from nine semen samples, recovered from the female partners of volunteer couples, and the sizes of the polymorphic DNA fragments were measured. These sizes were compared with those obtained from DNA purified from the blood of the male and female sexual partners. In all cases, the pattern of DNA polymorphisms obtained from the semen samples was identical to that of DNA purified from blood of the male sexual partner. This technique is applicable to the identification of an assailant in rape cases.

Introduction

In instances of sexual assault, the isolation of spermatozoa or prostatic acid phosphatase is the most common test used to indicate the occurrence of sexual activity (1), but yields no information concerning identity of the assailant. The analysis of a limited number of polymorphic proteins from semem recovered intravaginally or from dried stains is difficult and makes it hard to establish identity of an assailant to a high degree of certainty (2).

Differences in DNA nucleotide sequence among individuals can be visualized as size polymorphisms in restriction endonuclease digested DNAs. Because these restriction fragment length polymorphisms (RFLPs) have been shown to be inherited as Mendelian traits, they can be used as markers for genetic studies (3). Some RFLPs display a large number of discreet DNA fragments which greatly increase the chances for an individual possessing a distinct set of alleles.

This report describes the recovery of spermatozoa from dried semen stains, the purification of male-specific DNA from these samples, and restriction fragment length polymorphism analysis of this DNA, and DNA isolated from the blood of the male and female sexual partners.

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Materials and Methods

Sources of DNA

Matched blood and sperm samples were collected from volunteers. Blood was collected in Vacutainer tubes using potassium EDTA as anticoagulant and the semen was left untreated.

Nine dried semen samples deposited on women's undergarments or sanitary napkins following intercourse were obtained along with blood samples from the volunteer male and female sexual partners.

Isolation of DNA

Fabric containing dried semen was cut into small pieces and scrubbed with a small brush in 30 ml of phosphate-buffered saline (136 mM NaCl; 8 mM Na_2HPO_4; 17 mM NaH_2PO_4, pH 7.0), while sanitary napkins were processed by removing the plastic and cutting the absorbent material into small pieces. The pieces were soaked for 24 hours at 4° C in 100 ml of PBS containing 2.0% Sarkosyl. Solutions were filtered with nylon mesh to remove fabric, a sperm count taken, and the sperm heads pelleted by centrifugation at 5,000 x g for 10 minutes at 4° C. Fresh semen samples were resuspended in 20 ml of PBS containing 2.0% Sarkosyl, mixed briefly, and the sperm heads pelleted by centrifugation as above. Sperm heads were lysed by resuspending the pellet in 2.0 ml of PBS containing 2.0% Sarkosyl, 100 µg/ml Proteinase K, 10 mM DTT, and 25 mM EDTA, and incubated at 37° C for 20 hours with gentle rocking.

DNA was isolated from 5 to 10 cc of peripheral blood by lysing the red cells with 4 volumes of blood lysis buffer (0.32 M sucrose; 10 mM Tris-HCl, pH 7.6; 5 mM MgCl, 1.0% Triton X-100) followed by the pelleting of the white cells by centrifugation at 2,000 x g for 10 minutes at 4° C. The white cells were resuspended in 2 cc of DNA lysis buffer (10 mM Tris-HCl, pH 7.4; 10 mM NaCl, 10 mM EDTA), with Proteinase K and SDS added to 100 µg/ml and 1,0% respectively, and incubated 4 - 16 hours at 37° C with gentle mixing. Sodium perchlorate was added to 1.0 M to both blood and sperm DNA preps and the samples were either stored at 4° C or processed into DNA by extraction with phenol-chloroform (1:1) twice, chloroform twice, followed by dialysis against a 1000 fold excess of TE (10 mM Tris-HCl, pH 7.4; 1 mM EDTA). All DNA concentrations were measured in a spectrophotometer at 260 nm.

Restriction Endonuclease Digestion, Electrophoresis, and Hybridization

Two to five micrograms of DNA were digested with a five fold excess of the restriction endonucleases Eco Rl

or Taq 1 (Bethesda Research Laboratories) using conditions recommended by the manufacturer. Samples were routinely precipitated with ethanol after the first digestion, resuspended in 20 microliters, and loaded on agarose gels. Eco Rl digested samples were loaded in 0.4% agarose gels and electrophoresed 65 hours at 0.6 volts/cm while the Taq 1 digested samples were loaded in 1.2% agarose gels and electrophoresed at 1.8 volts/cm for 20 hours. The length of gels varied from 15 cm to 22 cm. After size fractionation by electrophoresis, DNA was transferred to nylon membranes, and hybridized with ^{32}P-labelled probes using standard procedures (4).

DNA Probes

Two probes were used in this investigation which recognize highly polymorphic regions in the human genome. pAW101 contains a 6.5 kilobase (kb) Eco Rl insert in pBR322 (kindly supplied by R. White). It hybridizes with the D14S1 locus located in chromosome 14 and is polymorphic with Eco Rl with over 30 alleles distributed from 14 to 32 kilobases (5). pLM0.8 contains a 879 base pair Cla 1 - Sph 1 insert in pBR322 and hybridizes to the 3'-flanking region of the HRAS-1 oncogene in chromosome 11 (6). It is polymorphic with Taq 1 with 18 distinct alleles from 1.8 to 4.5 kb.

Results and Discussion

DNA isolated from matched blood and semen samples was analysed using two probes, pAW101 and pLM0.8, which detect polymorphic regions. For each individual we compared the pattern of RFLP in both blood and semen DNA. We examined 106 Taq 1 digested matched DNA samples with pLM0.8 probe and 40 Eco Rl digested matched samples with pAW101 probe. No size differences between alleles were seen in the DNA isolated from the two tissues.

The number of sperm recovered from dried semen stains varied considerably, presumably due to natural variation in sperm count. The dried stains were from 1 to 7 days old before processing. Studies where a known amount of sperm was applied to cotton cloth and aged for one day to 8 weeks indicated that about 30% of the sperm are recovered by this procedure and was independent of sample age.

On average, the amount of DNA recovered from all samples was about 90% of the expected (number of sperm multiplied by 2.5×10^{-12} g). The average amount of DNA isolated from a dried semen stain was 40 µg.

Titration experiments indicate that the amount of DNA necessary for RFLP analysis varies from 1 to 4 micrograms. Thus the amount of DNA isolated from the dried stains was sufficient for multiple analyses in duplicate.

DNA isolated from all sperm samples was found to be high molecular weight. Each undigested DNA had a size larger than a 23 kb DNA size marker in agarose gels. This property is essential for RFLP analysis because degraded DNA consists of randomly cleaved DNA fragments that, after digestion and electrophoresis, do not migrate as discreet bands in a gel. As a result, degraded DNAs lose the radioactive bands normally seen after hybridization. The presence of bands larger than expected could result from incomplete digestion of DNA with restriction endonucleases. Thus, all DNAs were digested twice with at least a five fold excess of enzyme.

DNAs prepared from peripheral blood from the male and female volunteer sexual partners and from dried sperm recovered post-coitus, were used to prepare filters containing Eco Rl or Taq 1 digested DNA. These filters were hybridized with radioactively labelled DNA probes pAW101 or pLM0.8. In nine cases examined, the pattern of bands seen in the DNA lane from sperm matched the pattern of the DNA from the male peripheral blood. Thus, DNA purified from semen samples are not contaminated with detectable amounts of female specific DNA.

As with any polymorphic system, the ultimate power to assign identity depends on the number and frequency of alleles. We have collected a data base of over 1000 chromosomes for both probes from random blood donors in the New York City area. Information concerning race was used to assemble separate allele frequency tables. Presently, with the two probes used, the power of identity an individual is 1 in 1,000. The addition of more probes which recognize highly polymorphic DNA regions will increase the power of the DNA test.

Conclusions

Male specific high molecular weight DNA was isolated from dried semen stains up to 8 weeks old. The pattern of RFLPs seen with Eco Rl and Taq 1 digested DNAs hybridized with different probes which detect polymorphisms indicated the pattern seen with the semen DNA matched the male sexual partner. This procedure could prove useful in identification of assailants in rape cases.

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