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Human Population Genetic Studies of Five Hypervariable DNA Loci

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Summary

Population genetic studies were performed using DNA probes that recognize five hypervariable loci (D2S44, D14S1, D14S13, D17S79, and DXYS14) in the human genome. DNA from approximately 900 unrelated individuals, subdivided into three ethnic groups (American blacks, Caucasians, and Hispanics) were digested with *Pst*I and were successively hybridized to each DNA probe. The number of distinct DNA fragments identified for each of these regions varies from 30 to more than 80. An allele frequency distribution was determined for each locus and each ethnic group. The results show significant differences, between ethnic groups, in the pattern of distribution as well as in the relative frequency of the most common alleles of D2S44, D14S1, and D14S13 but only small differences in others (i.e., D17S79 and DXYS14). The results presented show that the analysis of these loci can have useful applications in population genetics as well as in identity tests.

Introduction

DNA polymorphisms arising from variations in the number of short tandem repeats may account for most of the hypervariable regions detected in the human genome (Nakamura et al. 1987*b*). Although a large number of such regions have been discovered, only a few of them have been extensively analyzed with regard to the number of different-size alleles detectable and their frequency distribution in different ethnic groups (Baird et al. 1986; Harumoto et al. 1988). For this study we have analyzed five such loci and have determined both the size range and frequency distribution of their alleles in American blacks, Caucasians, and Hispanics.

Material and Methods

DNA Isolation and Fractionation

DNA was prepared, from peripheral blood samples, as described elsewhere (Kanter et al. 1986). DNA was purified from blood samples received for paternity testing and provided by P. Rubinstein from the New York Blood Center and by J. Morris from the Paternity Testing Center, California. The samples represent individuals from the New York City and Los Angeles metropolitan areas. Family studies were done using nylon filters containing *Pst*I-digested DNA samples from the Centre d'Étude du Polymorphisme Humain (CEPH) families and from the Human Cell Repository (provided by M. Siniscalco). DNA (5 μg) was digested with a 10-fold excess of *Pst*I (Bethesda Research Laboratories [BRL]) according to the manufacturer's specifications. Fractionation of DNA was performed by electrophoresis in 0.9% agarose (BRL) gel (27 cm long) in recirculating TAN buffer (40 mM Tris hydrochloric acid, pH 7.9, 20 mM acetate, and 2 mM EDTA) for approximately 64 h at 0.8 V/cm. Following electrophoresis, DNA was transferred to nylon membranes (Micron Separations, Inc.) by using the capillary transfer method (Southern 1975; Maniatis et al. 1982).

DNA Hybridization

Radioactive DNA probes were prepared by the method of random primer extension (Feinberg and Vogelstein 1983) using [α-^{32}P] dCTP (specific activity >3,000 Ci/mmol; Amersham). Hybridization of DNA was done at 65°C for 16 h in a solution containing 0.75

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M sodium chloride, 0.05 M sodium phosphate, pH 7.0, 5 mM EDTA, 5% polyethylene glycol, 1% SDS, 100 μg of heparin (Sigma)/ml and denatured labeled DNA probe. Following hybridization, filters were washed at 65°C in 2 × SSC buffer (0.3 M sodium chloride, 0.03 M sodium citrate, pH 7.0, 0.5% SDS) followed by 0.1 × SSC.

Radioactive bands were visualized, by autoradiography at −70°C, with X-Omat AR (Kodak) X-ray film and Cronex Lightning Plus® (Dupont) intensifying screen. After the results were obtained, with a particular probe, radioactive DNA was stripped off the filter, by incubating in hybridization solution containing 50% formamide, at 65°C for 15 min and was rehybridized to a new probe.

DNA Probes

All probes used in this study recognize polymorphisms in *Pst*I-digested human DNA. The probe pAC061 was derived from an 800-bp unique-sequence DNA fragment, isolated by A. Wyman, cloned into pBR322. It is located approximately 2 kb from a hypervariable DNA sequence at the D14S1 locus (Wyman et al. 1986). The probe pAC225 or 29C1, isolated by H. Cook, contains short tandem repeats and recognized the locus DXYS14 (Cooke et al. 1985; Cooke and Smith 1986). The probe SLi103 consists of in vitro–ligated DNA fragments of the chemically synthesized consensus sequence of YNH24 (Nakamura et al. 1987*b*), and it recognizes the D2S44 locus (Lathrop et al. 1987). The probe pAC256 contains short tandem repeats and recognizes the D17S79 locus (McClain et al. 1987; Nakamura et al. 1987*c*). The probe pAC299 is a *Pst*I fragment cloned in Bluescript SK® (Stratagene) that recognizes the D14S13 locus (Nakamura et al. 1987*a*, 1987*b*). It was derived from the cosmid clone MLJ14 (ATCC # 57579).

RFLP Analysis

The size of the alleles, detected with each probe, was calculated by measuring their mobility relative to that of DNA fragments of known size. These measurements were done with a digitizing tablet connected to a computer. The general procedure used for these size determinations has been described elsewhere (Baird et al. 1986).

Allele Frequency Determinations

The frequency of the alleles, for each of the five loci described here, was determined using information generated from DNA samples prepared from parents received for paternity testing (see above). Racial information, for each sample, was provided by the submitting laboratory. The final results were subdivided into three ethnic groups (i.e., American blacks, Caucasians, and Hispanics) that were then analyzed separately.

Results and Discussion

General Properties of the Five Polymorphic Loci

The general properties of these polymorphic DNA loci were determined with DNA samples digested with *Pst*I. The probes used to identify these RFLPs contain the variable-number-of-tandem-repeat (VNTR) sequence or a synthetic multimer of the consensus sequence. The only exception is the probe (pAC061) used to detect the D14S1 locus. In this case, the DNA probe is a unique DNA sequence located approximately 2 kb from the hypervariable region. To date, the sequence responsible for this polymorphism has not been identified with certainty, although a candidate DNA sequence has been suggested (Wyman et al. 1986).

Since each of the probes used for these studies recognizes a single locus, the DNA of an individual has either two polymorphic fragment sizes (heterozygotes) or one (homozygotes). Four of the loci (D2S44, D14S1, D14S13, and D17S79) generate that type of pattern. At one locus, DXYS14, the number of polymorphic fragments can vary from one to six per individual. This locus contains, in each chromosome, between one and three closely spaced regions of VNTR separated by a spacer region up to a few thousand base pairs long. The restriction site(s), for the enzyme used to analyze the polymorphism, is located in the spacer region(s) between the VNTRs (Cooke and Smith 1986).

Evidence accumulated to date shows that probably most of the hypervariable regions contain VNTRs (Bell et al. 1982; Capon et al. 1983; Jarman et al. 1986). However, the number and size of the alleles can vary widely from locus to locus (Nakamura et al. 1987*b*; authors' unpublished observations). For the loci presented here, there are large variations in the size range of the DNA fragments detected (figs. 1–5). Under the conditions used to fractionate DNA, the sizes of the alleles vary by increments that are too small for us to visually distinguish them. As a result, the estimated number of alleles at each locus is a function of the size measurement error. As described elsewhere (Baird et al. 1986), for the experimental conditions used in these type of studies, the size measurement error (i.e., SD) is approximately 0.6% of the size of the allele. There-

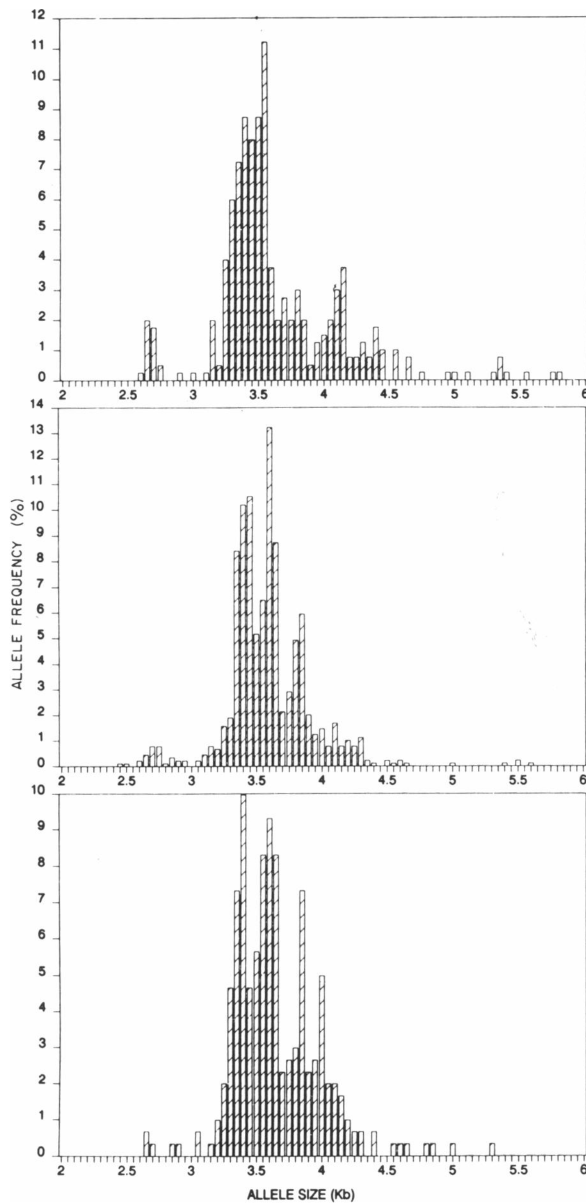

Figure 1 Allele frequency distribution of D17S79 alleles in *Pst*I-digested DNA from three American ethnic groups. The number of chromosomes analyzed for these figures were (*A*) 402 for American blacks, (*B*) 918 for Caucasians, and (*C*) 296 for Hispanics.

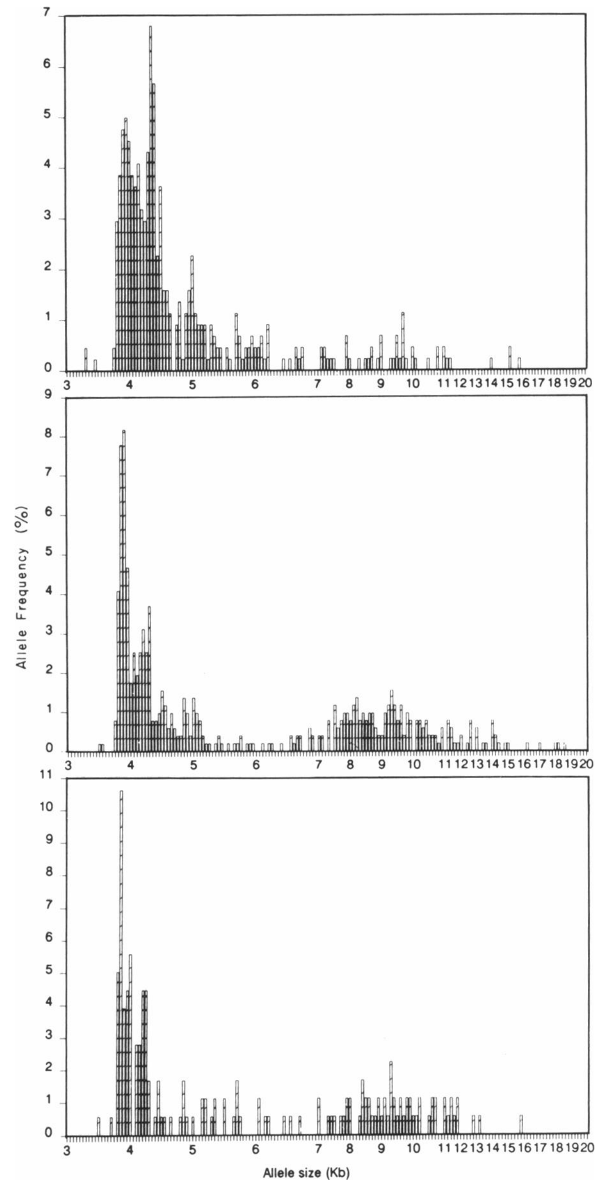

Figure 2 Allele frequency distribution of D14S1 alleles in *Pst*I-digested DNA from three American ethnic groups. The number of chromosomes analyzed for these figures were (*A*) 446 for American blacks, (*B*) 520 for Caucasians, and (*C*) 180 for Hispanics.

fore, two DNA fragments were considered to be of different size if their values differed by more than 3 SDs. As a result, we estimate that the number of distinct fragments at each locus varies from approximately 30 (D17S79) to >80 (D14S1 and DXYS14). Examples illustrating the banding patterns obtained with these polymorphic loci, in *Pst*I-digested DNA from random individuals, are shown in figure 6. The use of closely spaced molecular-weight markers provides the reference points required for the accurate measurement of the size of the alleles detected.

Family studies have shown that the alleles from all five loci segregate according to the laws of Mendelian inheritance. The frequency of homozygotes and heterozygotes detected for the various allele sizes was con-

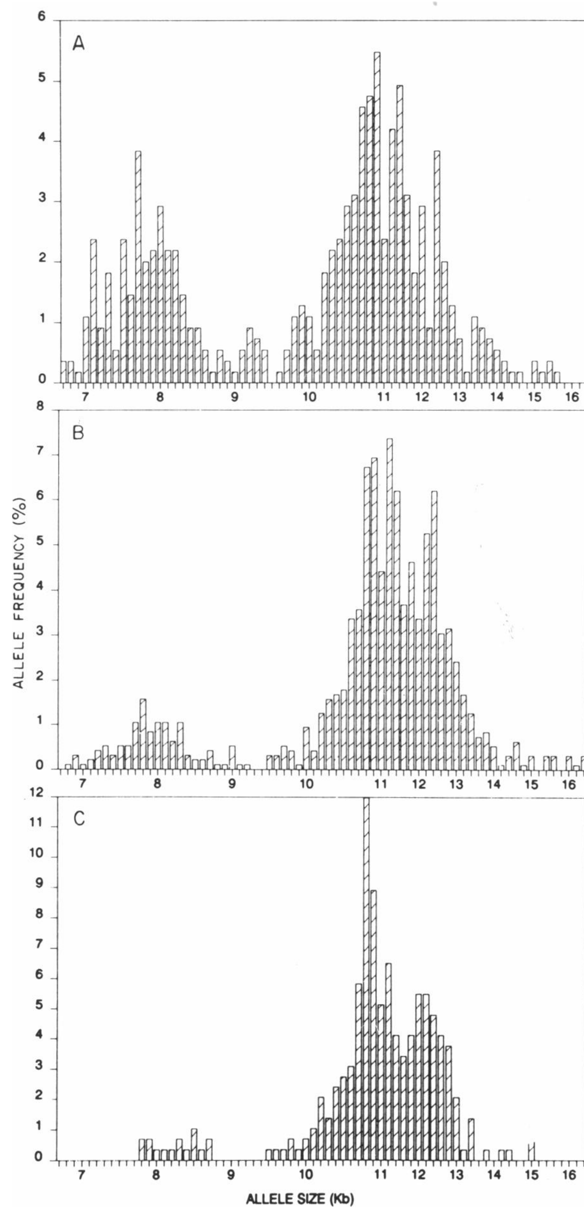

Figure 3 Allele frequency distribution of D2S44 alleles in *Pst*I-digested DNA from three American ethnic groups. The number of chromosomes analyzed for these figures were (*A*) 548 for American blacks, (*B*) 952 for Caucasians, and (*C*) 294 for Hispanics.

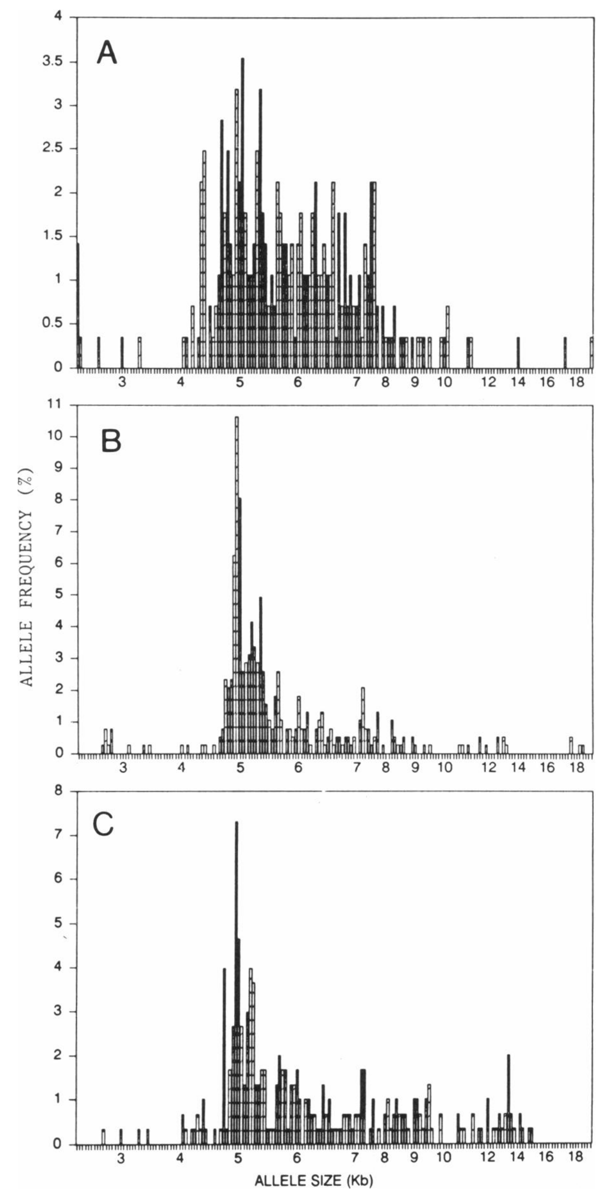

Figure 4 Allele frequency distribution of D14S13 alleles in *Pst*I-digested DNA from three American ethnic groups. The number of chromosomes analyzed for these figures were (*A*) 282 for American blacks, (*B*) 398 for Caucasians, and (*C*) 302 for Hispanics.

sistent with their being in equilibrium as calculated from their individual allele frequencies. Linkage studies have shown that D14S1 and D14S13 are at approximately 20 cM from each other (Nakamura et al. 1987*a*). No indication of gametic-phase disequilibrium was detected between the alleles of these two loci or between any of the loci presented here (results not shown).

It may be argued that some of these polymorphic loci contain alleles that are smaller than those retained by the gels used for these studies (i.e., alleles <1.2 kb or null alleles). The occurrence of such alleles would result in the detection of an excess number of homozygous individuals, relative to those predicted by the frequency of alleles. As mentioned earlier, no such excess of homozygotes has been detected. Another indication

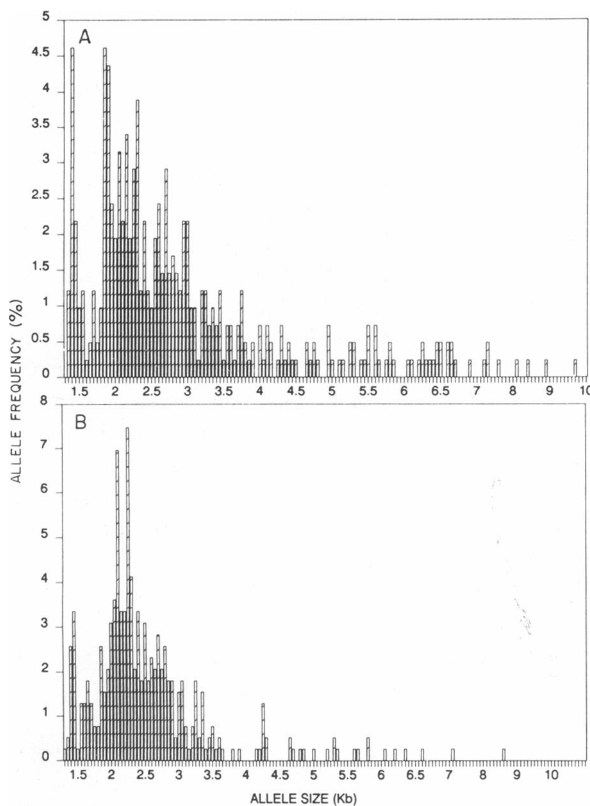

Figure 5 Frequency distribution of DXYS14 DNA fragments in *Pst*I-digested DNA from two American ethnic groups. The number of individuals analyzed for these figures were (*A*) 193 for American blacks and (*B*) 221 for Caucasians.

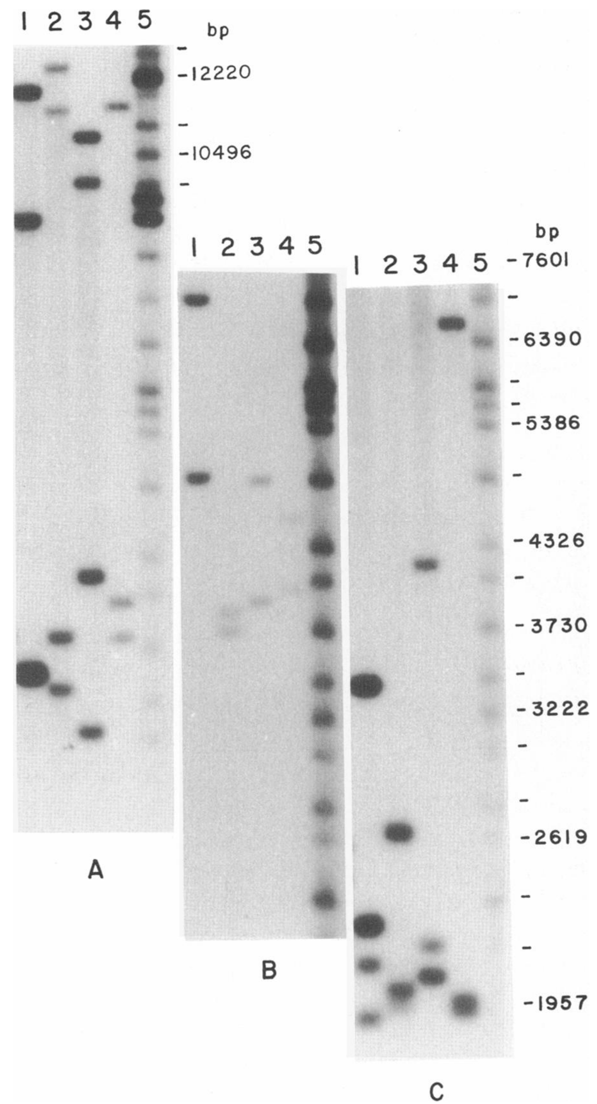

Figure 6 Examples of alleles observed in *Pst*I-digested DNA from four unrelated individuals for the loci (*A*) D2S44 [*top*] and D17S79 [*bottom*], (*B*) D14S1, and (*C*) DXYS14. The results in panels *A*–*C* were obtained from the same nylon filter, hybridized successively to one or two probes. Lanes 1–4 contain DNA from different individuals. Lane 5 contains size standards consisting of a mixture of bacteriophage λ and ΦX-174 DNA digested with various restriction endonucleases. The size of some of these DNA fragments is shown at the right of the photograph.

for the occurrence of null alleles would be the observation of cases of nonmaternity or of individuals that are homozygotes for null alleles. Family and paternity studies have not yet revealed such examples. Thus, if null alleles or alleles <1.2 kb exist, they are very infrequent.

It has been proposed that these VNTR-containing sequences are frequent sites for new mutations. This is based on the assumption that the large number of individual variations in the number of tandem repeats could arise by DNA rearrangements in the germ cells. The possibility of occurrence of recombinant alleles is important for the interpretation of paternity test results since they may lead to false exclusion of the alleged father or produce a maternal exclusion. Therefore, it is essential that cases of parental exclusion be confirmed by more than one locus. For example, for two genetic systems, each having a recombination frequency of 0.2%, the probability that both genetic systems would produce false exclusions in the same individual is only 1/500,000 cases, $1/(1/0.002 \times 0.002 \times 0.5)$. The detection of a rearrangement is determined by the resolution of the gel used for DNA fractionation. Therefore, the scoring of a recombinational event is an operational definition. A recombinational event can be scored only

if the new allele generated exceeds the minimum resolution of the gel. Under our experimental conditions DNA fragments within a 2% size range may appear to comigrate and are considered identical. To detect a recombinational event, the change in allele size — or the number of VNTR units gained or lost — has to increase in proportion to the size of the alleles. For example, in the 2.0-kb size range, DNA fragments have to differ by ⩾40 bp to be considered of different size, while, in the 8.0-kb size range, the difference has to be about 160 bp. Family studies have shown that the mutation rate of some hypervariable loci can be as high as 5% (Jeffreys et al. 1988). However, similar studies performed on the five loci described here has not revealed, with the exception of DXYS14 (two new mutations or 0.2% recombination), any meiotic recombination in approximately 1,000 gametes examined. The individuals examined represent about 600 meioses from the CEPH families and approximately 400 paternity tests examined for examples of nonmaternity.

Allele Frequency Distribution at the D2S44 Locus

On the basis of the frequencies of the various DNA fragment sizes, two major allele clusters were detected: one between 6.7 and 9.3 kb, with the most common allele at about 7.7 kb, and the other between 9.4 and 14 kb and with maximum frequency at about 10.7 kb (fig. 3). This general type of allele distribution was found in all three ethnic groups. However, a test of the null hypothesis that their total distributions are identical (Kolmogorov-Smirnov test) indicates that the distribution in American blacks is different from those of Caucasians or Hispanics. In addition, the fraction of the total alleles that was found in each cluster varies significantly within a particular ethnic group as well as between ethnic groups. For example, in American blacks the cluster of large alleles is 1.4 times more frequent than the small alleles, while in Caucasians and Hispanics the large alleles are 4.1 and 10 times more common, respectively. Also, the small-size alleles are 2.7 times more frequent in American blacks than in Caucasians. On the basis of the number of samples used for these studies, the calculated differences are all statistically significant ($P < .01$).

In addition to the analysis of allele frequency distribution in the three ethnic groups described above, we have further subdivided the samples into New York and California populations. Analysis of these two subpopulations for the D2S44, D17S79, and D14S1 loci did not show any statistically significant differences (results not shown).

Allele Frequency Distribution of the Major Polymorphism at the D14S1 Locus

The allele frequency distribution pattern described by Baird et al. (1986), on *Eco*RI-digested DNA, represents the sum of two known RFLPs: a minor (960-bp-insertion-deletion) and a major polymorphic (hypervariable) region at the D14S1 locus (Wyman et al. 1986). The allele frequency distribution obtained for the major polymorphism is shown in figure 2. Alleles have been detected for a size range from 3.5 to >18 kb. The most common alleles were found in the 3.7–4.5-kb size range, and they account for 50%–60% of the total alleles. However, as with the other polymorphisms described here, the size range of the alleles is very similar for all ethnic groups. Statistically significant differences in the allele frequency distributions were found only between American blacks and Caucasians or Hispanics. In the case of particular size ranges, such as 4.3–4.75 kb, the alleles are almost three times more common in American blacks than in Caucasians, while alleles of 6.55–12 kb are three times more frequent in Caucasians than in American blacks. All of these differences are statistically significant ($P < .01$).

The minor polymorphism consists mainly of two allele sizes (Baird et al. 1986), and they were found to be in equilibrium with the alleles of the major polymorphism (results not shown).

Allele Frequency Distribution at the D14S13 Locus

A statistical comparison of the frequency distributions of the three ethnic groups indicates that they are different from each other. The frequency distribution of the alleles present at this locus can be arbitrarily subdivided into three major size groups. The first group of alleles is 3–4.5 kb. The second is 4.55–5.5 kb, and the third is composed of alleles >5.5 kb (fig. 4). An examination of the allele frequency distribution between ethnic groups was performed by comparing the ratio of the first group of alleles to that of the second. The results show that the 3–4.5-kb:4.55–5.5-kb allele frequency ratio is approximately 1:4.5 in American blacks, 1:34 in Caucasians, and 1:8.5 in Hispanics. Also, the 3.0–4.5-kb alleles are about two to four times more common in American blacks than in Caucasians or Hispanics. All of these differences are statistically significant ($P < .01$).

Allele Frequency Distribution at the D17S79 Locus

The alleles identified for this locus are within a size range of approximately 4 kb, and most of the alleles are found distributed around the 3.4–3.6-kb DNA frag-

ment sizes. A comparison of the allele frequency distribution between ethnic groups shows some small qualitative differences (fig. 1). The only size range showing statistically significant differences between American blacks and Caucasians was 3.55–3.9 kb. That group of alleles is about 1.5 times more common in Caucasians and Hispanics than in American blacks.

Allele Frequency Distribution at the DXYS14 Locus

The analysis of this pseudoautosomal locus is complicated by the fact that the number of different DNA fragments at this locus can vary from one to three per chromosome. Family studies were used to determine the frequency of single-, double-, and triple-band haplotypes in American black and Caucasian populations. The most common haplotype consists of two sizes of DNA fragments (American blacks 55%, Caucasians 61%). The second most common haplotype contained a single fragment (American blacks 39.5%, Caucasians 32.1%). As a result, approximately 80% of individuals had a total of three to four different-size DNA fragments. The relative frequency of the one-, two-, or three-DNA-fragment haplotypes was not significantly different between American blacks and Caucasians. The size of the DNA fragments detected in the population was 1.3–9.0 kb. The frequency distributions of the DNA fragment sizes in American blacks and Caucasians is summarized in figure 5. The large majority of the DNA fragments were 1.4–3.5 kb. Statistical comparison of the frequency distribution of DNA fragments, between American blacks and Caucasians, show that both ethnic groups have the same general distribution. However, DNA fragments >3.55 kb are more than twice as frequent in American blacks as in Caucasians.

The combination of single-locus DNA polymorphisms offers a very powerful tool to solve genetic questions arising in such different areas of identity as parentage testing, forensic identification, and tissue engraftment (Ginsburg et al. 1985; Knowlton et al. 1985; Baird et al. 1986; Dykes et al. 1986; Giusti et al. 1986; Kanter et al. 1986; Wainscoat et al. 1987).

When the polymorphic loci described here were used for parentage testing, the average power of exclusion of each of these loci (table 1) was calculated using the formula described for RFLP by Ito et al. (1985). The allele frequencies used for these calculations were obtained by binning the alleles by the intervals shown in figures 1–4. The results indicate that the combination of these loci will exclude >99.99% of falsely accused individuals. In cases where an alleged father is included by these genetic systems and where a paternity index (PI) is calculated, the median combined PI is expected to be >1,000,000 (table 2). For identity tests, table 3 summarizes the discrimination potential of these loci. When the formula described by Fisher (1960) is used, the combination of these five loci will on the average distinguish $1/5 \times 10^{11}$ individuals. This value only serves to illustrate the power of these polymorphisms. The actual probability of occurrence of a particular combination of alleles will depend on their respective frequencies. For example, for an individual heterozygote for the most common alleles of these five loci, the likelihood of occurrence of that pattern in the population is approximately $1/5 \times 10^8$, while rarer alleles will generate higher values. Although family relatedness can result in shared alleles, this effect will disappear in a few generations. For example, when comparing two individuals from an extended pedigree who are separated by 4 generations of random mating, the probability of maintaining the same haplotype in one of the gametes for five hypervariable loci is <1/1,000,000 (i.e., $\{[0.5]^4\}^5$). If in these two individuals the other gamete is contributed by unrelated parents, the probability of being the same is $1/7 \times 10^5$ (i.e., $\sqrt{5 \times 10^{11}}$). Therefore, the probability that these two individuals, separated by 4 generations, will have the same genotype is more or less the same as that for unrelated individuals (i.e., $10^6 \times 7 \times 10^5 = 7 \times 10^{11}$). Of course, a number of scenarios are possible, depending on the particular pedigree under study, and calculations can be made accordingly. Alternatively, in the case of very close relatives, the simplest way to resolve the question of iden-

Table 1

Average Power of Exclusion of Five Hypervariable Loci for Unrelated Individuals

Ethnic Group	D2S44	D14S1	D14S13	D17S79	DXYS14	Cumulative
American blacks	.83	.81	.94	.81	.89[a]	.99996
Caucasians	.88	.70	.93	.79	.89[a]	.99996

[a] Empirical value.

Table 2

Median PI Obtained with Five Hypervariable Loci for Unrelated Individuals

Ethnic Group	D2S44	D14S1	D14S13	D17S79	DXYS14	Cumulative
Blacks	15	43	39	7	23	>1,000,000
Caucasians and Hispanics	10	39	20	9	23	

Table 3

Average Power of Identity of Five Hypervariable Loci for Unrelated Individuals

	MEAN LIKELIHOOD RATIO[a]					
ETHNIC GROUP	D2S44	D14S1	D14S13	D17S79	DXYS14	CUMULATIVE VALUE
American blacks	244	591	647	62	159	9×10^{11}
Caucasians	154	788	395	51	136	3×10^{11}

[a] Mean value for the genetic odds in favor of identity (indicates the chance that the identification is correct).

tity is by performing the DNA identity test on the suspected individuals. In summary, these results show that *Pst*I-digested DNA analyzed for five well-defined polymorphic loci can be an extremely powerful tool for identity testing.

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