1.3 Practical Use

Comparison of HLA Antigens and DNA Polymorphisms in Parentage Testing

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The power to exclude wrongly identified men involved in paternity disputes has been increased by recombinant DNA technology. A variety of human DNA probes have been cloned which can be used in Southern blot analysis to detect highly polymorphic loci within the genome of individuals. The probes that detect loci consisting of variable numbers of tandem repeats (VNTRs)appear to be especially useful in identity testing (Nakamura 1987). Of the 7 immunologic genetic tests that the joint American Medical Association - American Bar Association Committee recommended to establish non-paternity in routine investigations, the HLA system provides the greatest probability of excluding a wrongly identified man (Abbott et al. 1976). This article will describe the strategy we have taken for estimating the alleles in the population detected by use of VNTR probes as well as evalualting the power of HLA as opposed to DNA polymorphism testing for excluding wrongly identified men in paternity disputes.

The population investigated consisted of black and white paternity cases from Alabama who presented to the University of Alabama Health Services Foundation, Immunogenetics/DNA Diagnostic Laboratory for testing. All cases were assessed utilizing HLA-A and B typing and the VNTR probes YNH24 and PHINS 310 representing loci D2S44 and INS (CH. 11), respectively. The Promega GenePrint™ DNA typing protocol that was largely developed at the FBI Academy in Quantico, Virginia was followed for Southern blot analyses. The standards for DNA polymorphism testing of the American Society for Histocompatibility and Immunogenetics and The American Association of Blood Banks also were followed. Each electrophoretic gel run contained an internal allelic control consisting of DNA from an individual whose restriction fragment alleles were known as well as standard DNA fragment size markers that spanned the entire range of VNTR fragment sizes detected in the two populations. Fragment sizes were estimated by use of a digitizing pad connected to a PC programmed for autoradiograph reading and line fit. For a case consisting of a trio the DNA samples from the mother, child and putative father were loaded into the gel as well as a mixture of DNA from the child and putative father. In the event that the child's paternal fragment was similar in size to a fragment

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from the putative father the lane containing the DNA mixture was very informative in determining if indeed there was an exclusion.

Since VNTR probes detect highly polymorphic regions of the genome with the alleles often varying in size from one to a few hundred copies of the tandemly repeated sequence, it is important to establish the size range within which one can reproducibly measure a given fragment in the population. Based on blot to blot comparisons of the allelic control fragments in the 0.3 kb to 4 kb size range, fragments can be reproducibly measured within plus or minus 50 base pairs. However, due to the low frequencies of fragments greater than 4 kb we have arbitrarily made the operating assumption that one can only measure within plus or minus 400 base pairs which corresponds to pooling over several identifiable fragments. Based on these considerations we have established mathematically derived ranges of fragment sizes called bins within which the sample alleles can be grouped in order to calculate the allele frequencies. It should be noted that these bins may change in future as we obtain greater accuracy and reliability in measuring fragments over the entire size spectrum.

RESULTS

Utilizing YNH24 and Hae III restriction endonuclease we have observed fragments that range in size from 0.2 kb to 6.4 kb in the black population and 0.8 kb to 8.8 kb in the white population from Alabama. Using size bins which span either 100 base pairs for fragments in the range of 0.2 kb to 4 kb or 400 base pairs for fragments greater than 4 kb, 44 alleled can be assigned within a sample of 153 blacks and 41 alleles within a sample of 156 whites for the YNH24 probe (Table 1). The size range of fragments detected by PHINS 310 and PVU II restriction endonucleases range from 0.6 kb to 4.7 kb in the black population and 0.5 kb to 4.6 kb in the white population. As can be seen in Table 1 the polymorphic information content (PIC) is higher for the loci detected by the YNH24 probe in the white population (Botstein 1980). The PIC for each of the two loci is less in the black population than for the white population. The YNH24 probe has the greatest exclusion probability. This probe has the power to exclude approximately 94% and 85% wrongly identified black and white men, respectively.

Table 1. Power of VNTR probes to exclude falsely accused individuals

Probe	Enzyme	Race	Sample N	Alleles N	PIC %	Exclusion Probability %
PHINS 310	PVU II	W	262	31	92.2	74.9
		B	100	30	89.4	69.0
YNH24	Hae III	W	156	41	94.7	93.9
		B	153	44	86.7	85.0

Using the YNH24 allelic frequencies that have been estimated for the Alabama population we have compared the power of DNA polymorphism testing to HLA antigen testing in resolving 58 white and 81 black cases of paternity. We were able to exclude with both HLA and YNH24, 21 white and 33 black putative fathers.

However, 9 white and 6 black putative fathers were excluded only by YNH24. In comparing the paternity index (PI) obtained by use of HLA and YNH24 in 28 white and 42 black non-excluded cases, respectively, the PI was higher with HLA in 15 white and 25 black cases. In contrast the PI obtained by use of YNH24 was higher in 13 white and 17 black cases. Although, we do not have as much case work experience with use of PHINS 310 as compared to YNH24, based on the population frequencies of alleles detected by these two VNTR probes the cumulative exclusion probability would be approximately 98.5% in whites and 95.4% in blacks.

SUMMARY

The VNTR probes evaluated provide a powerful system for resolving disputed cases of paternity. However we feel that the population sampled is not adequate to detect all the alleles that may exist in the population. We are presently attempting to increase our population database to include at least 500 white and 500 black individuals. In addition, it will be important to compare the allele frequencies detected by these probes in whites and blacks from various geographic regions. It is known that the degree of white admixture in the black population varies from one region of the USA to another (Reed 1969). Thus, it will be essential that regional databases of allelic frequencies be established for paternity case work. When adequate databases of allelic frequencies for the various racial and ethnic groups represented in the USA becomes available, DNA typing should be a single technique with sufficient power to resolve most disputed cases of paternity.

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