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Restriction Site Polymorphism (RFLP) Detected with pG3PD and Taq 1.

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INTRODUCTION

Blood banks have been performing genetic testing since the discovery of the ABO blood groups by Landsteiner in 1900. Today blood banks routinely test for a number of clinically relevant blood groups and some also determine HLA phenotypes for tissue transplantation purposes. In addition, some blood banks perform genetic testing to evaluate cases of disputed parentage. Genetic markers that are analyzed in paternity cases include blood group and HLA antigens determined by standard serological methods and red cell enzymes and serum proteins analyzed by electrophoretic methods. Each of the testing methods is distinct and requires a different expertise.

RFLP (Restriction Fragment Length Polymorphism) mapping represents one of the newest methods of genetic testing. In this technique, chromosomal DNA is digested with an endonuclease specific for a particular sequence of nucleotide bases which cuts the DNA into a heterogenous family of fragments differing in size and separable by electrophoresis in agarose gels. Individual fragments within the digest can be visualized following their transfer from the agarose gel onto filter paper using Southern blotting (Southern 1975) and then hybridizing the filter to a cloned DNA probe that has been labeled in some way. The application of RFLP mapping to genetic testing relies upon the polymorphisms that exist within the population in the presence or absence of a particular restriction endonuclease recognition site in the chromsomal DNA. A single nucleotide base substitution in the chromosomal DNA can create or destroy a restriction site thus altering the size of a DNA fragment in the digest. Restriction site polymorphisms are inherited in Mendelian fashion and thus fulfill the criteria for a genetic marker. The power of RFLP mapping relates to the fact that essentially all of the chromosomal DNA can be analyzed with the technology whereas the classical methods analyze only the 1% or so of the DNA that actually encodes and expresses a gene product. Thus, a much larger pool of genetic information is available for evaluation.

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We have been involved in studies aimed at evaluating the feasibility of applying RFLP mapping to routine paternity testing. As part of our effort, we have been systematically testing a variety of cloned cDNA fragments for their ability to detect RFLPs in samples of chromosomal DNA obtained from random blood donors. We recently reported the isolation of a cloned cDNA encoding the enzyme glyceraldehyde 3-phosphate dehydrogenase (pG3PD) (Allen et al 1987). We have used this cloned probe to determine if RFLPs exist in those regions of the genome that hybridize to the pG3PD probe. We report here that pG3PD does detect an RFLP generated with the enzyme Taq 1.

MATERIALS AND METHODS

Chromosomal DNA was isolated from buffy coats of 10 ml blood samples obtained from the donor population within our blood service region. Nuclei were obtained from white cells during a 60 minute incubation of the buffy coat on ice in 5 mM Tris-Cl pH 7.5 containing 2.5 mM $MgCl_2$, 160 mM sucrose, and 0.5% Triton X-100. Nuclei were pelleted from the lysate by centrifugation. Chromosomal DNA was liberated from the nuclei during an overnight incubation at 37° C in 400 ul of 50 mM Tris-Cl pH 7.5 containing 24 mM EDTA, 75 mM NaCl, 1% SDS and 100 ug/ml proteinase K. Chromosomal DNA was extracted with phenol:chloroform (9:1) and then twice with chloroform:isoamyl alcohol (24:1). DNA was then dialyzed against several changes of 10 mM Tris-Cl pH 8.0 with 1 mM EDTA at room temperature to remove residual traces of organic solvents. DNA concentration was estimated on the basis of U.V. absorption at 260 nm.

Ten microgram aliquots of chromosomal DNA were digested in a 200 ul volume with 20 units of Taq 1 (Bethesda Research Labs) at 65° C for 4 hours as recommended by the supplier. DNA fragments were recovered from the digest by ethanol precipitation and centrifugation and then were electrophoresed in 0.8% agarose gels in 89 mM Tris-borate buffer pH 8.2 containing 2 mM EDTA. Following electrophoresis, gels were soaked for 10-15 minutes in 0.25 M HCl to depurinate the DNA and facilitate transfer. The gels were then soaked and blotted onto Zetabind membranes (AMF Cuno Meriden, CT) in 0.4 N NaOH. The filters were rinsed in 0.1X SSC at 65° C for 30 minutes and then air dried. Filters were pre-hybridized overnight at 42° C in 50% de-ionized formamide, 20 mM PIPES pH 6.8, 0.8 M NaCl, 2 mM EDTA, 0.1% SDS, 5X Denhardts solution, 200 ug/ml sheared, denatured salmon sperm DNA, 10 ug/ml poly A, and 10% (wt./vol.) dextran sulfate. Hybridization was performed for 20-24 hours at 42° C in the same buffer containing 5 X 10^6 cpm/ml of nick translated and heat denatured insert from the pG3PD plasmid. Blots were washed 4 times at room temperature for 10 minutes each in 2X SSC + 0.1% SDS, 2 times at 65° C for 30 minutes each in 1X SSC + 0.1% SDS, and 2 times at 65° C for 30 minutes each in 0.1X SSD + 0.1% SDS. Filters were exposed for 24-72 hours to X-ray film in cassettes containing intensifying screens.

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RESULTS

Numerous restriction fragments present in Taq 1 digests of chromosomal DNA hybridized to the pG3PD probe (Fig. 1) in keeping with the large number of G3PD sequences present in the human genome (Benham et al 1984, Hanauer and Mandel 1984, Tso et al 1985). The majority of these fragments were invariant in digests of random samples of chromosomal DNA. However, a Taq 1 polymorphism is also present in the digests representing a simple two allele system with fragments migrating with apparent sizes of either 3.9 kb or 2.7 kb (Fig. 1). The intensity of hybridization of the probe to the polymorphic fragments varies depending upon the genotype of the particular individual. Thus, in individuals homozygous for one of the alleles, the polymorphic band is roughly twice the hybridization intensity of the same band in a heterozygous individual (Fig. 1).

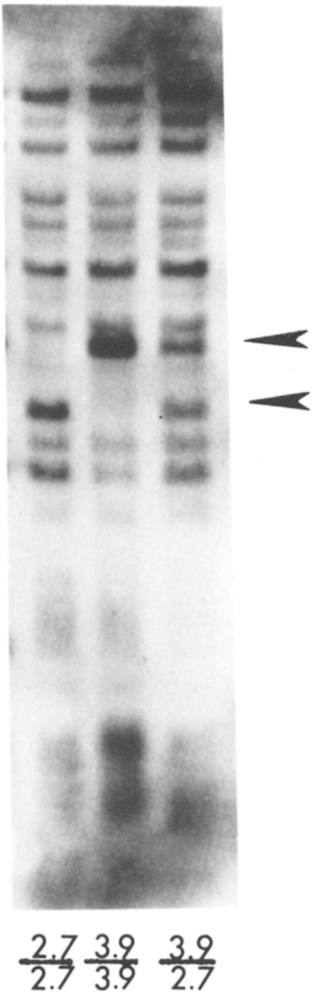

Figure 1 -- Hybridization of the pG3PD cDNA to a Southern blot of chromosomal DNA digested with Taq 1.

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Aliquots of chromosomal DNA were digested wiith Taq 1 electrophoresed in agarose, blotted onto a nylon membrane and hybridized to nick translated insert from the pG3PD plasmid as described in Materials and Methods.

The gene frequencies of the different alleles are 0.745 for the 3.9 kb allele and 0.245 for the 2.7 kb allele based on the analysis of over 100 random samples of chromosomal DNA. These gene frequencies also hold for a more limited sampling of chromosomal DNA obtained from blacks. The Taq 1/pG3PD RFLP is passed in families as an autosomal marker in a Mendelian fashion (Fig. 2).

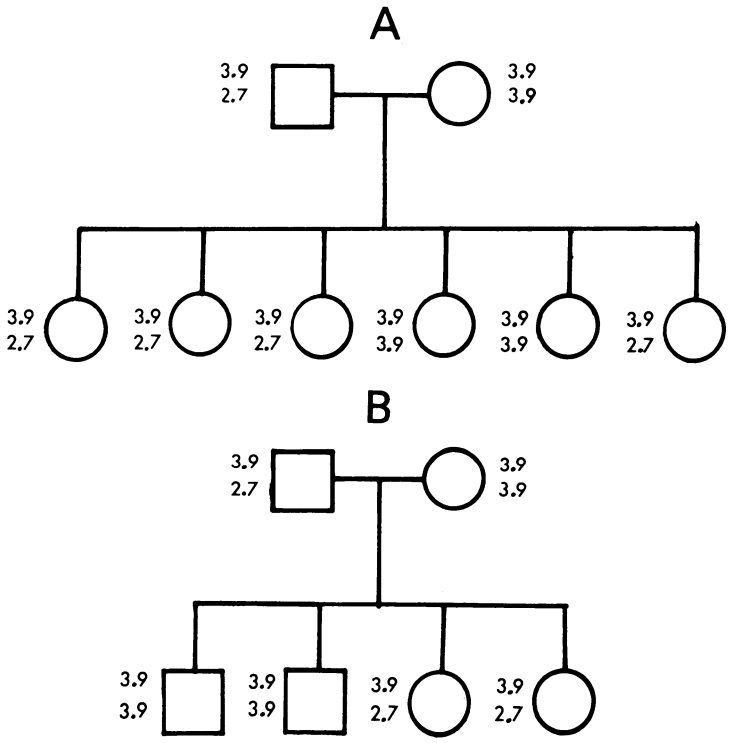

Figure 2 -- Mendelian inheritance of the pG3PD/Taq 1 RFLP in two families.

RFLP analysis was performed as in Figure 1 using chromosomal DNA from family members. Circles represent females and squares males in the figure.

The chromosomal location of pG3PD/Taq 1 RFLP was determined by analyzing chromosomal DNA from human-mouse somatic cell hybrids (kindly provided by Dr. F. Ruddle, Yale University) containing a limited number of human chromosomes. Results indicate that the RFLP is located on chromosome 12 (not shown).

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DISCUSSION

Using a full length cDNA encoding the enzyme glyceraldehyde 3-phosphate dehydrogenase we have identified an RFLP in the human genome generated with the restriction enzyme Taq 1. This RFLP represents a simple two allele system with restriction fragments of either 3.9 kb or 2.7 kb present in Taq 1 digests of chromosomal DNA. The frequencies of the two alleles is 0.745 for the 3.9 kb allele or 0.255 for the 2.7 kb allele in over 100 random samples analyzed. The RFLP is passed in Mendelian fashion as an autosomal trait and analysis of somatic cell hybrids has localized the RFLP to chromsome 12. The functional gene encoding the G3PD enzyme has been mapped to chromosome 12 using somatic cell hybridization techniques (Bruns and Gerald 1976).

Multiple gene sequences encoding G3PD have been found in the human genome (Benham et al 1984, Hanauer and Mandel 1984, Tso et al 1985). The one presumed functional gene has been localized to chromsome 12 (Bruns and Gerald 1976) however, other sequences have been found on the X chromosome (Benham et al 1984, Hanauer and Mandel 1984). The sequence(s) on the X chromosome were found to exhibit characteristics of a non-functional pseudogene and thus remain silent. The Taq 1/pG3PD RFLP must be either located within an intron region in the functional G3PD gene or be located within a region closely flanking the gene that is not present in G3PD gene sequences located on other chromosomes since the RFLP behaves as a single genetic trait.

We are presently searching for other restriction enzymes that generate RFLPs detectable with the pG3PD probe. Since a large number of G3PD related gene sequences exist within the genome, it may be possible to identify RFLP on other chromosomes that would segragate independently of the Taq 1 RFLP and make the pG3PD probe a more informative tool in our paternity testing program.

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