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Variable Number of Tandem Repeats in Zygosity Diagnosis in Twins

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Abstract. The use of DNA restriction fragment length polymorphisms (RFLP) to analyze variable number of tandem repeat (VNTR) sequences dispersed in the human genome, has become a powerful tool for the study of population genetics due to the very substantial polymorphism involved. Because the markers usually employed for twin zygosity determination (such as sex combination, placentation, HLA typing, blood group antigens, etc) may not be uniformly informative, we propose the use of synthetic olygonucleotides, representing VNTR " core " sequences, for the determination of zygosity in twins.

Key words: Restriction fragment length polymorphism, Twin zygosity

INTRODUCTION

Although numerous genetic and anthropologic markers are available for determining zygosity in twins, there is still a need for a more practical and informative method in zygosity diagnosis.

Studies on genetic variation have been enhanced in recent years by the analysis of restriction fragment length polymorphisms (RFLP). Several hundred probes detecting RFLP have been characterized: most probes originate from systematic searches of randomly cloned human DNA fragments or from chromosome specific libraries. These probes, used as genetic markers, are able to distinguish a single base variation in a single copy sequence.

The human genome contains unique and repetitive sequences, the latter organized in sequences where a core stretch is repeated in a variable number of copies. Tandem repeat sequences are interspersed in the human genome showing a high degree of polymorphism. The family of repetitive sequences is composed by two major groups: the minisatellite group, serially repeated at few chromosome sites, and the VNTR randomly spread in the human genome.

Because of the number and frequencies of their alleles, these regions of repetitive DNA are regarded as very informative genetic markers. We have therefore evaluated the feasibility of developing a double synthetic oligonucleotide probe system to assess twin zygosity.

MATERIAL AND METHODS

As a preliminary approach to the problem a blind study was conducted on 7 twin pairs, the zygosity of which was assessed using, in sequence, blood groups, HLA typing, and DNA analysis with the following techniques.

DNA Extraction. To speed up results, we used a rapid DNA extraction procedure, starting from 0.5-3 ml of peripheral blood samples. Briefly, buffy coats of nucleated cells obtained from anticoagulated blood were resuspended in 10 mM Tris-HCl, 400 mM NaCl, 1% SDS, pH 8.2. The cell lysates were digested overnight with protease K (0.3 mg/ml of cell lysate). After the digestion was complete, 1 ml of 6 M NaCl was added to each sample, mixed and centrifuged. Two volumes of precooled absolute ethanol were used to precipitate the high molecular weight DNA.

Enzyme Digestion. 3-5 μg of purified DNA were digested with restriction enzyme Hinf-I (Toyobo, Japan) at the final concentration of 10 units per μg of DNA.

Oligonucleotide Probes. Oligonucleotides were synthetized on a Beckman DNA synthetizer. The YNH-24 probe derives from a VNTR core sequence in the insuline gene region [1], and the 33.1 probe derivates from Jeffrey's minisatellites [2]. Probe sequences YNH-24, 5′-TCCTGAACAACCCCACTGTACTTCCCA-3′; 33.1, 5′-GTGCCTGCTTCCCTTCCCTCTTGTC-3′.

Direct Gel Hybridization. The digested DNA was electrophoresed on a 0.8% agarose gel for 16 hr at 40 V. The gel was then denatured, neutralized and vacuum dryed on a standard device. 20 picomoles of ^{32}P-5′ end labeled oligonucleotide probes were used for each hybridization.

Blood Grouping and HLA Typing. Standard laboratory methods were used for blood grouping. Serological HLA typing was done according to the NIH standard protocol [3].

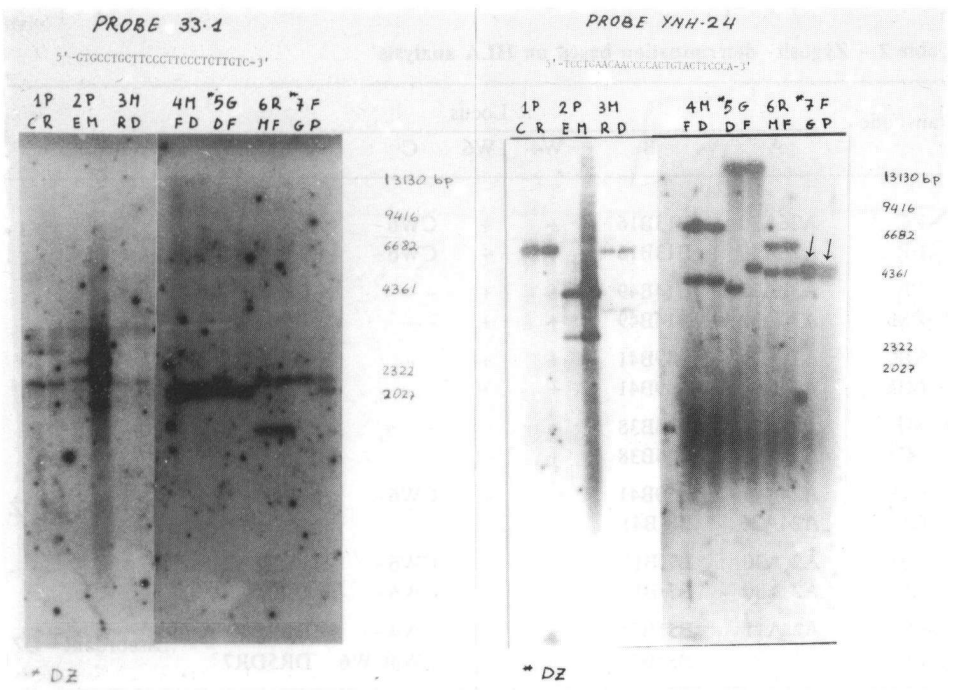
RESULTS AND DISCUSSION

AB0 and Rh blood group analysis (Table 1) shows 1 pair to be discordant, and therefore DZ, the remaining 6 pairs being concordant. HLA typing (Table 2) shows 2 pairs to be discordant, and therefore DZ (one of them being the same already found to be DZ by AB0 blood grouping). Thus, after two tests, we find that 5 out of 7 pairs can be assumed to be MZ.

The same result can be obtained with a single DNA probe. The 33.1 oligonucleotide probe hybridizes with different fragments in 2 twin pairs (Figure, **a**), the remining pairs all showing a concordant hybridization pattern. This is also found with the YNH-24 oligonucleotide probe (Figure, **b**).

It should be considered that HLA typing, though more informative then AB0 and Rh blood grouping, is time consuming and relatively expensive since a reliable typing needs an expanded set of antisera (not to mention the fact that HLA identical siblings are seen quite often, as it happens, eg, in transplantations).

In contrast, the use of synthetic oligonucleotide probes derived from the highly polymorphic repetitive sequences, VNTR and minisatellites, is nowdays the fastest and most reliable tool in twin zygosity determination.



(a) **(b)**

Figure. Hybridization pattern of probes 33-1 (**a**) and YNH-24 (**b**). Different fragments found in twin pairs 5 and 7, thus diagnosed as DZ. The slight difference between fragments in pair 7 (see arrows in **b**) is due fo few " core " sequences (3-5 repetitive units).

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Table 1 - Zygosity determination based on blood group analysis

Pair code	ABO	Rh	
1–PC	A2	ccDuE2	
1–PR	A2	ccDuE2	
2–PE	B	CcDEe	
2–PM	B	CcDEe	
3–MR	B	CcDee	
3–MD	B	CcDee	
4–MF	B	CcDEe	
4–MD	B	CcDEe	
5–GD	A1	CcDee	Discordant = DZ
5–GF	A1B	Ccadee	
6–RM	B	CCDee	
6–RF	B	CCDee	
7–FG	B	CcDee	
7–FP	B	CcDe	

Table 2 - Zygosity determination based on HLA analysis

Pair code	Locus						
	A	B	W4	W6	C	DR	
1–PC	A26A30	B13B16	+	+	CW6–	nd	
1–PR	A26A30	B13B16	+	+	CW6–	nd	
2–PE	A30A32	B14B49	+	+	– –	DR1DRW6	
2–PM	A30A32	B14B49	+	+	– –	DR1DRW6	
3–MR	A1 A2	B49B41	+	+	– –	nd	
3–MD	A1 A2	B49B41	+	+	– –	nd	
4–MF	A2 A24	B44B38	+	–	– –	DR2–	
4–MD	A2 AD4	B44B38	+	–	– –	DR2–	
5–GD	A24 –	B50B41	–	+	CW6–	DR5–	Discordant = DZ
5–GF	A24A26	B8 B41	–	+	– –	DR3DR5	
6–RM	A2 A30	B7 B13	+	+	CW6–	DR2DR7	
6–RF	A2 A30	B7 B13	+	+	CW6–	DR2DR7	
7–FG	A2 A11	B51B35	+	+	CW4–	DR5–	Discordant = DZ
7–FP	A1 A2	B35B37	+	+	CW4CW6	DR5DR7	

We plan to extend our screening to a larger panel of twins, to control the reproducibility of the method and to study the rate of spontaneous mutations. These could involve either the restriction sites flanking the repetitive sequences, or the gain or loss of a sub-

stantial number of repeated units, which is consistent with length changes arising primarily from unequal exchange at meiosis. Germline instability should be taken into account when using hypervariable loci as genetic markers.

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