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The Use of Microsatellites in Zygosity Diagnosis of Twins

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Abstract. Although numerous genetic and anthropological markers are available for determining zygosity of twins, there is still a need for a more practical and informative method in zygosity diagnosis. Dinucleotide repeats or other short repeats (microsatellites) are highly variable between individuals and offer a simple, fast, cheap, and exact approach for zygosity determination. The feasibility of a set of microsatellites to be used for this purpose is demonstrated.

Key words: Twins, Zygosity diagnosis, Polymerase-chain-reaction (PCR), Highly polymorphic genetic markers, Dinucleotide repeats.

INTRODUCTION

The determination of zygosity is a prior task in twin research before its utilization in any kind of genetic investigation. Conventionally, the zygosity of a set of twins has been determined by blood group analysis and serological markers. These methods are reliable but rather troublesome and expensive and cannot be applied immediately after birth. DNA fingerprinting with multilocus minisatellite DNA probes has also been used for zygosity determination [2,5]. Incomplete digestion of the DNA may lead, however, to additional bands and so identical (MZ) twins could in this eventuality be falsely regarded as fraternal (DZ) twins [3]. Due to the lack of a clear genetic model such artefactitious bands are not reliably recognized as such.

Recently, several hypervariable, single locus variable number of tandem repeat (VNTR) markers were applied for zygosity determination [1]. The VNTRs were analyzed by the Southern Blotting method. Using information on single copy sequences flanking the repeats also allows for typing of VNTRs by use of the polymerase chain reaction (PCR). Neverthe-

less, the length of fragments to be amplified, as well as the size range of alleles, makes the optimization of PCR conditions mandatory for the different markers.

The use of microsatellites is a fast and easy-to-handle alternative to determine twin zygosity. Short, simple, repetitive sequences of the form $(dC\text{-}dA)_n \cdot (dG\text{–}dT)_n$ constitute one of the most abundant human repetitive DNA families. These sequences exhibit length polymorphisms and represent a rich source of highly informative genetic markers [9]. They can be typed with the application of PCR and single-copy-primers flanking the $(CA)_n$–repeats. The shortness of the amplified fragments (70–200 bp) allows for PCR performance under simple, standard conditions without need of time-consuming optimization of protocols. Efficiency in typing the microsatellites can be improved further by amplifying several loci simultaneously in the same tube and on the same gel. Thus, microsatellites offer a simple, fast, cheap, and precise approach for zygosity determination. In this study, we present data at 8 loci in order to demonstrate the feasibility of the method. Allele frequencies were determined in 40 unrelated Caucasians of German origin (Table 1).

MATERIALS AND METHOD

DNA extraction

Venous blood samples, anticoagulated with EDTA, were drawn from all probands. DNA was extracted from buffy coat preparations by salting out with saturated NaCl-solution.

PCR

Standard polymerase-chain-reaction was carried out in a 25 μl volume containing 40ng genomic DNA template, 5 pmol of each oligodeoxy nucleotide primer, 200 μmol each of dGTP, dTTP, dATP, 20 μmol dCTP and 0.75 μCi α^{32}dCTP at 800 Ci/μmol and 50 mmol/1 KCl, 10 mol/1 Tris "Cl" (pH 8,3), 1.5 mmol $MgCl_2$, 0.01% gelatine and 1 unit of Taq-Polymerase (BRL). Samples were overlaid with mineral oil and processed in a Perkin-Elmer Cetus DNA Thermal Cycler through 27 temperature cycles consisting of 1 min at 94°, 2 min at 55° and 2 min at 72°. The last elongation step was lengthened by 7 minutes. The primers for two loci were run simultaneously as a multiplex PCR (D1S117 and D4S171, D8S85 and ASS, D19S47 and D10S89, D9S43 and D5S107) [6,9-15].

Primer sequences

Oligonucleotides were synthetized by MWG Biotech, Ebersberg. Primer sequences are given in Table 2.

Electrophoresis and autoradiography

Samples of the amplified DNA were mixed with 2 Vol of formamide sample buffer and subjected to electrophoresis on a standard, denaturing, polyacrylamide DNA-sequencing gel. After vacuum-drying, the gels were exposed overnight. Sizes of the alleles were determined in accordance with the M13mp18 DNA sequencing ladders. The most intense band for each allele on the gels was used to obtain allele size (see Figure).

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Table 1 - Allele frequencies at the loci D4S171, D1S117, ASS, D8S85, D10S89, D19S47, D9S43 and D5S107 determined in 40 unrelated Caucasians

	Allele size	Allele frequency	Allele size	Allele frequency
	D4S171		*D1S117*	
A1	163bp	0.03	128bp	0.03
A2	161bp	0.02	126bp	0.04
A3	159bp	0.08	124bp	0.09
A4	157bp	0.02	122bp	0.24
A5	155bp	0.07	120bp	0.31
A6	153bp	0.22	118bp	0.09
A7	151bp	0.37	116bp	0.06
A8	149bp	0.03	114bp	0.03
A9	147bp	0.10	112bp	0.05
A10	145bp	0.01	110bp	0.04
A11	143bp	0.01	108bp	0.01
A15	135bp	0.01	100bp	0.01
A16	133bp	0.03		
	D8S85		*ASS*	
A1	84bp	0.01	147bp	0.04
A2	82bp	0.32	145bp	0.14
A3	80bp	0.13	143bp	0.11
A4	76bp	0.32	141bp	0.07
A5	74bp	0.21	139bp	0.08
A6			137bp	0.11
A7			135bp	0.06
A8			133bp	0.39
	D10S89		*D19S47*	
A1	156bp	0.03	106bp	0.00
A2	154bp	0.04	104bp	0.03
A3	152bp	0.13	102bp	0.15
A4	150bp	0.15	100bp	0.27
A5	148bp	0.06	98bp	0.07
A6	146bp	0.09	96bp	0.05
A7	144bp	0.49	94bp	0.11
A8	142bp	0.01	92bp	0.31
A9			90bp	0.01
	D9S43		*D5S107*	
A0	104bp	0.01	155bp	0.02
A1	102bp	0.01	153bp	0.07
A2	100bp	0.00	151bp	0.11
A3	98bp	0.23	149bp	0.29
A4	96bp	0.31	147bp	0.21
A5	94bp	0.12	145bp	0.02
A6	92bp	0.20	143bp	0.13
A7	90bp	0.05	141bp	0.02
A8	88bp	0.04	139bp	0.01
A9	86bp	0.03	133bp	0.12

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Table 2 - Primer Sequences

Marker locus	Primer sequences[1]
D4S171	5'TGGGTAAAGAGTGAGGCTG3'
	5'GGTCCAGTAAGAGGACAGT3'
D1S117	5'CCTTTTGCCTCCTTCGT3'
	5'CTCATTTACAATAGCTACC3'
D8S85	5'AGCTATCATCACCCTATAAAAT3'
	5'AGTTTAACCATGTCTCTCCCG3'
ASS	5'AGGTCCGAAAACACAAAG3'
	5'GGGAGCTATAAAAATGACCA3'
D10S89	5'AACACTAGTGACATTATTTTCA3'
	5'AGCTAGGCCTGAAGGCTTCT3'
D19S47	5'GATGTCTCCTTGGTAAGTTA3'
	5'AATACCTAGGAAGGGGAGGG3'
D9S43	5'TTCTGATATCAAAACCTGGC3'
	5'AAGGATATTGTCCTGAGGA3'
D5S107	5'GATCCACTTTAACCCAAATAC3'
	5'GGCATCAACTTGAACAGCAT3'

[1]The CA primer strand is listed first for each twin pair

RESULTS AND DISCUSSION

A continuously growing number of highly polymorphic microsatellites is available for zygosity diagnosis. When the parents' genotypes are unknown, the probability of a dizygotic twin pair to be concordant depends on the number of alleles and their frequencies in the population [8]. The probability that a DZ twin is concordant for the two alleles is

$$P(\text{conc/DZ}) = \{[1 + \sum_{i=1}^{N} p_i^2]^2 + \sum_{i \neq j}^{N} (p_i p_j)^2\}/4$$

where p_i is the frequency of the i-th allele in a system containing N alleles [6]. If the marker systems are independent, the probability for a pair of DZ twins to be concordant and same-sexed can be calculated:

$$P_c = P(\text{conc and same sexed/DZ}) = 0.5 \times P_{c1} \times P_{c2} \times \ldots \times P_{cK}$$

Sex is treated as a marker with a concordance probability of approximately 0.5. For the proposed set of 8 microsatellite systems the combined probability is 0.0002 (Table 3). From this combined probability one can calculate (using Bayes-Theorem) the *a posteriori* probability to be MZ for twins that are same-sexed and concordant for the marker systems:

$$P(\text{MZ/conc and same sexed}) = \frac{1}{1 + (Q \times 0.5 \times P_{c1} \times \ldots \times P_{cK}}$$

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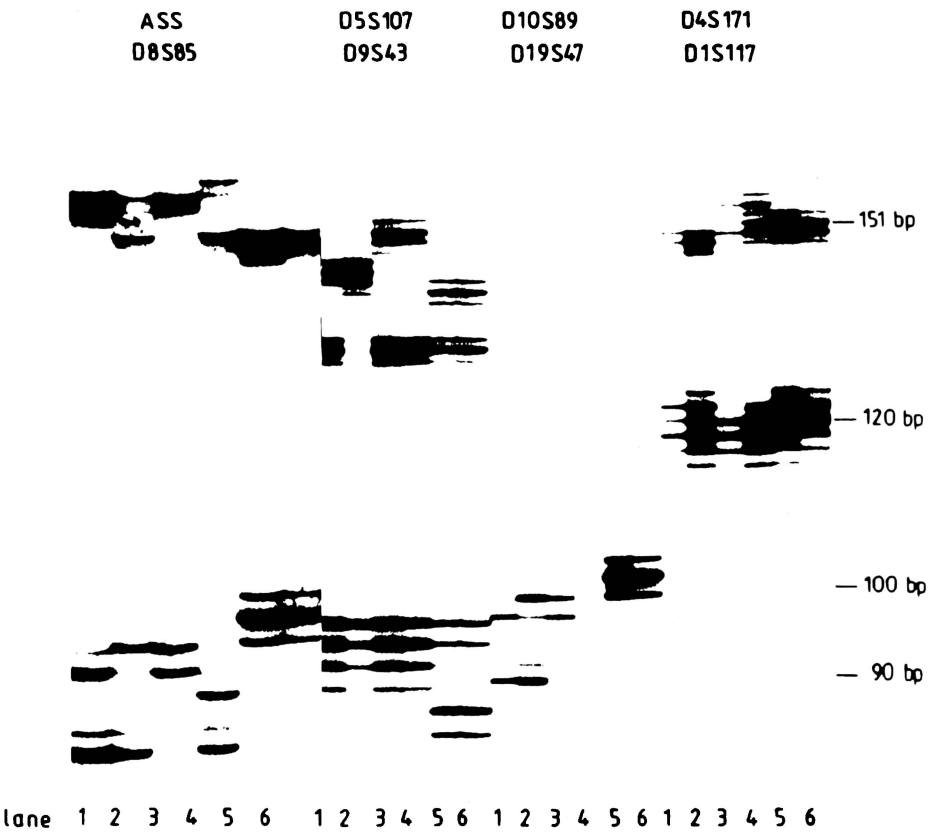

Figure. PCR products after electrophoretic separation at the D10S89 and the D19S47 locus, the D4S171 and D1S171 locus, the ASS and D8S85 locus and the D9S43 and the D5S107 locus, respectively, in 3 twin pairs: lane 1 + 2 = DZ, lane 3 + 4 = DZ, lane 5 + 6 = MZ (bp = base pairs)

Genotypes: *ASS* A2/A2, A2/A5, A2/A2, A1/A5, A5/A6, A5/A6
D8S85 A3/A5, A2/A5, A2/A3, A4/A5, A1/A1, A1/A1
D5S107 A5/A10, A5/A5, A1/A10, A1/A10, A7/A10, A7/A10
D9S43 A3/A4, A3/A4, A3/A4, A3/A4, A3/A7, A3/A7
D10S89 A3/A3, A3/A6, A3/A3, A2/A6, A6/A7, A6/A7
D19S47 A4/A7, A3/A7, A3/A4, A5/A7, A2/A2, A2/A2
D4S171 A5/A6, A6/A6, A2/A5, A2/A5, A4/A5, A4/A5
D1S117 A3/A5, A3/A5, A4/A5, A4/A5, A3/A4, A3/A4

where Q (= 1,5 in Germany) is the *a priori* ratio of DZ/MZ in the population [4]. For the eight marker systems used the probability is 99.97%.

Since for any given twin pair, probability will depend on whether they are concordant for common or rare alleles and whether the frequencies of the different alleles at each loci used vary up to 30-fold, the actual probability of monozygosity may be signifi-

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Table 3 **Probability that a DZ twin is concordant for the loci D4S171, D1S117, ASS, D8S85, D10S89, D19S47, D9S43 and D5S107, respectively. The combined probability for concordance in DZ twins of the same sex includes a factor of 0.5, because approximately 50% of DZ twins are opposite sexed.**

Marker locus	P(conc/DZ)	P(conc and same-sexed/DZ) combined
D4S171	0.3720	0.186
D1S117	0.3538	0.066
ASS	0.3728	0.025
D8S85	0.4125	0.010
D10S89	0.4254	0.004
D19S47	0.3747	0.002
D9S43	0.3726	0.0006
D5S107	0.3523	0.0002

cantly different from the probabilities calculated above. In addition, therefore, we calculated the probability for a same-sexed twin pair to be monozygotic when concordant for the most frequent allele combination of every marker system. This probability is 99.85%. For most twin studies in medical genetics this probability is high enough to be accepted. In practice, the marker loci are, of course, stepwise examined. As soon as discordance is observed at one locus, the twin pair are inevitably regarded as dizygotic. In determining zygosity, the number of marker loci used depends on the level of reliability the investigator wishes to achieve.

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