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How Many Markers or How Many Alleles per System are Appropriate in Zygosity Testing?

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A B S T R A C T

Due to the development in DNA-PCR-technique more and more systems with a high number of alleles have been established in twin diagnosis. Because of their high effectiveness in resolving of genetic questions it is not amazing that some authors have postulated the thesis that typing of 5 to 10 DNA-PCR systems can prove monozygosity. For this paper the use of different systems (conventional and PCR systems) has been tested for twin diagnosis and the observed effects are discussed.

Introduction

Due to the increasing number of DNA--PCR-polymorphisms and the higher degree of multiple allelism than in classical systems[1,2] the use of these polymorphisms is accepted not only in paternity testing but also in twin diagnosis[3,4]. The information capacity of DNA-PCR systems in twin diagnosis however had been overestimated sometimes[5]. This paper shows the effect of varying allele numbers and varying number of systems on the results of twin diagnosis.

Material and methods

Using a test excluding the parents[6,7] the probabilities of monozygosity with varying allele frequencies and varying number of systems have been calculated. Furthermore, in some cases the influence of including data of the parents[3,4] has been tested too. Concrete results of monozygosity testing in three cases are demonstrated. Since actual twin cases were not available, three paternity cases (probability of paternity according to Essen-Möller > 99.9999%) had been used by assuming the child being »twins« of same sex.

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Results and discussion

Tables 1–3 show the results of some selected genotype combinations of twins and their parents. In tables 1–2 theoretical polymorphisms has been used, consisting of different alleles with comparable gene frequencies and same information content of the systems. As can be seen from the tables, the quality of results in twin diagnosis seems to depend less on the frequency of the alleles than on the number of systems typed. The profit of information rises from 0.4871 (p = 0.9000) to 0.6315 (p = 0,0200), whereas it rises from 0.5783 (1 system studied, p = 0.5000) to 0.999991 (25 systems studied, p = 0.5000, table 1). However if the allele frequencies considered are relatively high (table 3, p = 0.9000) the results are scarcely satisfactory. Therefore polymorphisms like ADA, AK, PGD, ESD, etc. are not well suited for twin diagnosis. But even in those cases the number of polymorphisms is more decisive. The advantage of polymorphisms with more alleles (DNA-PCR systems) is due to the fact that allele frequencies higher than 0.5000 are rare.

Clearly these constructed cases show only the tendency of the problem. Table 4 shows the results of the calculated examples. The panel covers 26 polymorphisms including 14 DNA-PCR systems (case 1), 25 / 14 systems in case 2) and 23 / 11 systems in case 3, resp. The results of all systems (parents not included) demonstrate a high level of probability for monozygotic

TABLE 1
PROBABILITY OF MONOZYGOSITY WITH VARYING ALLELE FREQUENCIES AND VARYING NUMBER OF SYSTEMS. GENOTYPES OF TWINS PQ, GENOTYPES OF PARENTS PQ / PQ OR P^2 / PQ

	Parents not included					Parents included
Number of alleles	2 alleles (0.5000 each)	5 alleles (0.2000 each)	10 alleles (0.1000 each)	20 alleles (0.0500 each)	50 alleles (0.0200 each)	
1 system	0.5783	0.6224	0.6293	0.6310	0.6315	0.6316
5 systems	0.8999	0.9575	0.9631	0.9644	0.9648	0.9648
10 systems	0.9895	0.9983	0.9987	0.9988	0.9989	0.9989
15 systems	0.9989	0.99994	0.99996	0.99996	0.99996	0.99996
20 systems	0.99990	0.999998	0.999998	0.9999990	0.9999990	0.9999990
25 systems	0.999991	0.99999991	0.99999996	0.99999996	0.99999996	0.99999997

TABLE 2
PROBABILITY OF MONOZYGOSITY WITH VARYING ALLELE FREQUENCIES AND VARYING NUMBER OF SYSTEMS. GENOTYPES OF TWINS P^2, GENOTYPES OF PARENTS PQ / PQ OR PQ / PR. THE TRIPLET: PARENTS P^2 / P^2, TWINS P^2 IS NOT INFORMATIVE.

	Parents not included					Parents included	
Number of alleles	2 alleles (0.5000 each)	5 alleles (0.2000 each)	10 alleles (0.1000 each)	20 alleles (0.0500 each)	50 alleles (0.0200 each)	Parents pq / pq or pq / pr	Parents p^2 / pq
1 system	0.6038	0.7042	0.7391	0.7567	0.7672	0.7742	0.6316
5 systems	0.9384	0.9930	0.9971	0.9981	0.9986	0.9989	0.9648
10 systems	0.9963	0.99996	0.999993	0.999997	0.999998	0.9999990	0.9989
15 systems	0.9998	0.9999997					0.99996
20 systems	0.999990		All cases: > 0.9999999				0.9999990
25 systems	0.9999993						

TABLE 3
PROBABILITY OF MONOZYGOSITY WITH VARYING ALLELE FREQUENCIES AND VARYING NUMBER OF SYSTEMS. GENOTYPE OF TWINS: p^2

Allele frequencies	p = 0.9000	p = 0.8000	p = 0.7000	p = 0.6000
1 system	0.4871	0.5141	0.5426	0.5725
5 systems	0.5887	0.7108	0.8132	0.8887
10 systems	0.7051	0.8758	0.9567	0.9867
15 systems	0.7997	0.9529	0.9912	0.9986
20 systems	0.8696	0.9830	0.9983	0.9998
25 systems	0.9176	0.9940	0.9997	0.99998

TABLE 4
THREE EXAMPLES OF MONOZYGOSITY TESTING. IN DEFAULT OF TWIN CASES, THREE CONCRETE PATERNITY CASES HAD BEEN USED, ASSUMING THE CHILD BEING »TWINS«.

	Case 1			Case 2			Case 3		
	»Twins«	Mother	Father	»Twins«	Mother	Father	»Twins«	Mother	Father
ABO	A1	A1	A1	0	0	B	0	0	0
RH	ccD.Ee	ccD.Ee	Ccddee	CcD.ee	CcD.ee	CCD.ee	ccD.ee	ccD.ee	ccD.ee
P1	p	p	P	P	P	P	p	p	P
BF	S	S	FS	FS	FS	FS	S	S	S
C3	F	FS	F	S	S	S	S	S	S
HPA	2–1	2–1	2				2–1	2–1	2–1
PI	M1	M1	M1	M1	M3–M1	M3–M1			
ACP	AC	BC	AB	B	BC	B	B	B	AB
ADA	1	1	1	1	1	1	1	1	1
ESD	1	1	1	1	1	1	2–1	2	1
GLO	2–1	2	1	1	1	1	2–1	1	2–1
PGM1	a2a1	a2a1	a3a1	a2a1	a3a1	a2	a2a1	a2a1	a1
CD4	5–6	6–6	5–5	6–10	6–6	6–10			
D8S1132	20–20	20–25	18–20	18–19	18–20	19–19			
F13B	8–10	8–9	10–10	9–9	9–9	9–10	6–8	6–9	8–10
FES	10–12	10–12	10–12	9–9	9–9	9–11	11–11	11–12	11–11
FGA	19–25	22.2–25	19–20	19–24	21–24	19–24	21–23	21–22	20–23
TH01	6–7	7–8	6–7	8–9.3	8–9	9.3–9.3	9–9.3	8–9	8–9.3
VWA	17–17	16–17	17–19	15–17	15–19	17–18	17–17	17–17	16–17
D1S80	24–25	24–25	24–31	24–31	18–31	18–24	24–29	28–29	24–28
HLA-DQ?	1.1–1.2	1.1–4	1.1–1.2	1.2–2	1.2–2	1.1–1.2	1.1–1.2	1.2–4	1.1–2
LDLR	AB	AB	AB	AB	BB	AA	AA	AB	AA
GYPA	AA	AA	AB	AB	AB	AB	AB	BB	AB
HBGG	AB	AA	BB	AA	AA	AA	AB	AA	BB
D7S8	BB	BB	BB	BB	BB	BB	AA	AB	AA
GC	2–1S / AC	2 / AA	1S / CC	1S / CC	1F-1S / BC	1S / CC	1S / CC	1F-1S / BC	1S / CC

TABLE 4 (continued)

	Probability for monozygotic twins		
	Parents not included		
All systems	99.99998%	99.99990%	99.9996%
DNA-PCR systems only	99.9896%	99.9929%	99.93%
	Parents included		
All systems	99.99998%	99.99989%	99.99994%
DNA-PCR systems only	99.998%	99.996%	99.998%

twins. However, in case 1 and case 2, the results of the DNA-PCR systems alone seem to be not sufficient for a reliable twin diagnosis. If the parents were included, the calculations resulted in probabilities of more than 99.99% for all systems and for the DNA-PCR systems too. Though some polymorphisms proved to be not informative in the calculations including the parents (case 1 nine, case 2 ten and case 3 seven systems), the results in all cases were better than in those without parents.

The results demonstrate a combination of lower and higher information in the polymorphisms studied. The thesis is confirmed that satisfactory results can only be obtained with a minimum of 15 to 20 polymorphisms studied. To the contrary, the idea sometimes discussed that typing of 5 to 10 polymorphisms would be sufficient to prove monozygosity must be rejected. In clinical cases of medical treatment, for example transplantation of bone marrow, such an approach can lead to lethal incidents.

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KOLIKO JE MARKERA ILI KOLIKO JE ALELA PO SISTEMU POTREBNO ZA TESTIRANJE ZIGOTNOSTI

S A Ž E T A K

Zbog razvoja DNA –PCR tehnike ustanovljeno je sve više sustava sa velikim brojem alela u bizigotnoj dijagnostici. Zbog njihove visoke učinkovitosti u rješavanju genetičkih pitanja, nije začuđujuće da su neki autori postavili tezu da tipiziranje 5 do 10 DNA – PCR sustava može dokazati monozigotnost. U ovom radu ispitana je uporaba različitih (konvencionalnih i PCR) sustava za dijagnostiku bizigotnosti.