DNA PCR POLYMORPHISMS IN PATERNITY TEST PROTOCOLS. A BIOSTATISTICAL APPROACH

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INTRODUCTION

Over the years there has been a continuous increase of the number of genetic polymorphisms which have become available for paternity testing. The range has extended from the red blood cell groups, through serum proteins and erythrocyte enzymes polymorphisms to white cells antigens of the HLA system. With these "classical" markers, the use of several laboratory techniques is needed to achieve a sufficiently high standard of efficiency both in paternity exclusion and inclusion.

In the last few years the analysis of DNA polymorphisms has been routinely introduced into the forensic haemogenetics laboratories. Due to the very high level of variability and to the great number of these novel polymorphisms, just one well defined technique (e.g.: Southern blot hybridisation of multilocus minisatellites, single locus profiling, PCR essay) could be regarded as an exhaustive way to investigate a paternity relationship. As a consequence, also the variability among protocols adopted in different laboratories is considerably increased .

Until 1991, the Forensic Haemogenetics Laboratory of the University of Pisa used, in disputed parentage cases, six blood cell groups (AB0, RH, MNS, K, FY and JK), nine serum protein systems (HP, GC, TF, PI, C3, BF, PLG, IGHG and IGK) and eight red cell enzyme polymorphisms (ACP1, PGM1, AK, ADA, 6PGD, GPT, ESD and GLO). Since then, we included PCR essay. Currently, we use seven DNA polymorphisms: DQA1, D1S80, YNZ22, APOB, TH01, VWA and FES. Shortage of manpower resources and cost-benefit considerations discourage us, a small laboratory, from the routinary typing of 30 different systems for every paternity case.

In this paper our criteria of choice of polymorphisms are discussed. The biostatistical evaluation of efficiency in paternity exclusion plays a predominant role.

MATERIALS AND METHODS

Allele frequencies of DQA1, APOB, D1S80, YNZ22, TH01, VWA and FES polymorphisms were estimated from adequate population samples (n=110-165) of healthy individuals born in Tuscany (APOB: see Domenici *et al* 1994a, TH01: see Domenici *et al* 1994b, FES: see Tagliabracci *et al* 1995; other polymorphisms: unpublished data). The average power of exclusion (A) for the PCR-based systems has been calculated according to Garber and Morris (1983).

The data about allele frequencies of the 23 classical polymorphisms in Italy and their average power of exclusion were found in Piazza *et al* (1989) and in Bargagna and Domenici (1988).

Statistical parameters of the 7 PCR-based polymorphisms have been compared to those of the 23 traditional genetic markers. In this regard three parameters were taken into account:

cum $A\geq 1$ = cumulative chance of finding at least a single incompatibility
cum $A\geq 2$ = cumulative chance of finding at least two incompatibilities
cum $A\geq 3$ = cumulative chance of finding at least three incompatibilities

The last two indexes have been obtained by an iterative calculation, using the equations:

$$\Delta\text{cum } A\geq 2 = A\ (\text{cum } A\geq 1 - \text{cum} \geq A2)$$

$$\Delta\text{cum } A\geq 3 = A\ (\text{cum } A\geq 2 - \text{cum}\geq A3)$$

where $\Delta\text{cum}A\geq 2$ and $\Delta\text{cum}A\geq 3$ are the increase of power of exclusion for each new system that is added to the analysis, and A in the power of exclusion of the new system.

RESULTS AND DISCUSSION

Table 1 shows the allelic frequencies of DQA1, D1S80, APOB, YNZ22, TH01, VWA and FES in Tuscany, and their average power of exclusion (A). The mean value of A, in these polymorphisms, is about 60%. Only in FES system A is less than 50%. In contrast, the higher A value in classical systems is 33% of PGM1 and HP subtypes. Moreover, only 7 out of 23 traditional systems (RH, MNS, HP, GC, PI, ACP1, PGM1) have A higher than 25%.

Table 1: Allelic frequencies and average power of exclusion of seven PCR-based systems

DQA1	n = 110	**TH01**	n = 165	**VWA**	n = 110	**FES**	= 162
1.1	.154	6	.239	14	.091	10	.287
1.2	.186	7	.182	15	.105	11	.358
1.3	.082	8	.136	16	.209	12	.278
2	.146	9	.155	17	.282	13	.068
3	.086	9.3	.261	18	.241	14	.009
4	.345	Rare	.027	19	.064	Rare	.000
				Rare	.008		
A =	**.595**	A =	**.599**	A =	**.599**	A =	**.445**

APO B	n = 128	**YNZ22**	n=165		**D1S80**	n = 148	
31	.074	1	.061	14	.007	28	.044
33	.070	2	.164	16	.003	29	.057
35	.266	3	.118	18	.162	30	.014
36	.008	4	.270	19	.003	31	.078
37	.363	5	.061	20	.020	32	.014
39	.070	6	.030	21	.034	34	.007
41	.012	7	.021	22	.037	35	.007
43	.004	8	.033	23	.010	36	.007
45	.012	9	.103	24	.429	37	.007
47	.047	10	.073	25	.027	40	.003
49	.066	11	.021	26	.020	>41	.010
51	.008	12	.039				
		13	.003				
		14	.003				
A =	**.583**	A =	**.775**		A =	**.596**	

Table 2: The cumulative power of exclusion of different combinations of systems

# Systems	**cum A≥1**	**cum A≥2**	**cum A≥3**
23 (Traditional)	99.10%	94.22%	81.79%
7 (PCR-based)	99.87%	98.30%	90.77%
30 (Overall)	99.999%	99.98%	99.82%
20 (Selected)	99.996%	99.93%	99.48%
14 (Selected)	99.99%	99.82%	98.75%

Table 2 shows individual an overall values of cumA≥1, cumA≥2 and cumA≥3 in the traditional and PCR-based polymorphisms. Even if the 7 DNA polymorphisms appear to be more informative than the 23 traditional ones, either the first ones or the second ones alone could be regarded as a self-sufficient way to investigate a paternity relationship, on the ground of cumA≥1 values. Nevertheless, its as been a hallmark of paternity testing that in any situation scientists have always preferred to obtain evidence of non-paternity on more than one test system (for a recent review on criteria for paternity investigations, see: Mayr and Rossi 1993). So, cumA≥2 seems to be a better parameter than cumA≥1 for assessing the efficiency of conventional systems to provide evidence of non-paternity. By using our combination of traditional systems, it is expected that in about 6% of parentage cases involving non-fathers further investigation is needed.

As far as DNA systems are concerned, we feel that - in the present state of insufficient knowledge about their mutation rates - at least three incompatibilities should be obtained as a solid proof of paternity exclusion.

Table 2 shows that in about 10% of cases of non-paternity it is not possible to achieve three incompatibilities by using only the 7 PCR-based systems.

The whole repertoire of 30 test systems (both conventional and DNA) would satisfy our rather conservative standards (see table 2), albeit with an unfavourable cost-benefit rate. Comparable figures can be obtained through our present protocol, including 20 selected systems (AB0, RH, MNS, HP, GC, TF, PI, C3, ACP1, PGM1, ESD, GLO, GPT, DQA1, D1S80, APOB, YNZ22, TH01, VWA and FES). Also a more time and money saving protocol, based on 14 systems only - exclusively chosen on the ground of biostatistical criteria (A>25% as a threshold: RH, MNS, HP, GC, PI, ACP1, PGM1, DQA1, D1S80, APOB, YNZ22, TH01, VWA and FES) - would be appropriate. But we prefer to keep some redundancy in the number of conventional polymorphisms. This allows to obtain in the large majority of non-paternity cases (97%, in our protocol) incompatibilities in both traditional and DNA systems, and also to preserve the technical abilities acquired by laboratory staff over many years.

The goal of any forensic haemogenetics laboratory is to produce results that could not to be reasonably questioned in court trials. In our opinion, this means that we should reach either a probability of paternity higher than 99,8-99,9% or 2-3 genetic incompatibilities. CumA≥1 is the key biostatistical parameter to evaluate the efficiency of test systems used both in exclusion and in inclusion (the mean value of probability of paternity [W] is mathematically related to cumA≥1: see Nijenhuis 1979 and Morris 1983), but in our experience also cumA≥2 and cumA≥3 proved to be useful in choosing the more convenient protocol.

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