Int J Legal Med (2002) 116:64–67
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ORIGINAL ARTICLE

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Power of exclusion revisited: probability of excluding relatives of the true father from paternity

Received: 4 August 2000 / Accepted: 7 January 2001

Abstract In parentage testing using DNA markers, the formulae for calculating the probability of exclusion generally overstate the power of a test battery by considering its ability to exclude a random man. It is known that in many cases, in particular immigration applications, the false father is more likely to be a relative, e.g. brother, of the true father than an unrelated man. This work presents formulae that take this consideration into account. A practical example using Hong Kong data is provided to illustrate the effect of the modification. Also discussed is how the expected efficacy of a test battery will be affected when possible mutations and null alleles or genetic inconsistencies are taken into consideration.

Keywords Power of exclusion · Kinship coefficients · PCR · STR

Introduction

The effectiveness of a genetic marker as a tool for resolving paternity disputes is generally measured by its ability to exclude false fathers. The exclusion probability calculation was first described by Wiener et al. (1930) on two-allele systems. General formulae for systems with any number of codominant alleles were developed subsequently (Ohno et al. 1982; Garber and Morris 1983). The quantities computed by these formulae, however, can only reveal the power of a system to exclude a random man rather than a relative of the true father as the biological father. Unlike illegitimacy cases, the false father in inheritance or immigration disputes would be less likely to be an unrelated man, but rather a relative of the true father, e.g. his brother. Since this man has a high chance of sharing genes with the true father, it is harder to exclude him from paternity than an unrelated man. Knowing the power of a panel of tests to exclude a relative of the true father from paternity can help a parentage testing laboratory to interpret results more properly so as not to make undue claims to establishing the paternity of a particular child. Salmon and Brocteur (1978) derived the probability of excluding paternity when there were specific relationships between the mother, the biological father and the accused man by using the I-T-O method (Li and Sacks 1954). The probability they calculated, however, was only based on two-allele systems. With the utility of RFLP and/or PCR/STR tests, markers tend to have several to many codominant alleles. It would therefore be convenient to have general formulae for estimating the probability of excluding relatives of the true father from paternity for systems with any number of alleles.

This paper is organised as follows: the first section reviews the general formulae calculating exclusion probabilities for with-mother and no-mother cases. The second section derives the formulae for calculating the probability of excluding a specific relative of the true father from paternity. It is found that these formulae bear a simple relationship and the differences are illustrated in the following section using allele frequency data for 12 STR loci for the Hong Kong Chinese.

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Probability of exclusion

Consider a genetic system with n codominant alleles A_1, A_2,•••,A_n with their corresponding allele frequencies being p_1, p_2,•••,p_n. Given the genotypes of a child and the mother being G_C and G_M respectively, the probability of

exclusion for a particular mother-child genotype combination or the individual probability of exclusion (IPE) is the proportion of random men that can be excluded from being the father of this child, based on G_C and G_M.

Consider, at a particular locus, that the mother and child have genotypes $G_M = A_1A_2$ and $G_C = A_1A_1$, respectively. Any man without the A_1 allele will be excluded as the father of the child. In this case, $(1-p_1)^2$ of the whole male population can be excluded and hence $IPE = (1-p_1)^2$, i.e.

$Pr(\text{a random man is excluded}|G_M, G_C) = (1-p_1)^2$.

The probability of this mother-child combination is given by:

$Pr(G_M = A_1A_2, G_C = A_1A_1) = p_1^2 p_2$.

The IPE for the other mother-child combinations and the corresponding probabilities can be derived similarly (Ohno et al. 1982). The power of exclusion (PE) is then obtained by summing the IPEs for all the mother-child combinations, weighted by the corresponding probabilities:

$$\begin{aligned} Q_1 &= \sum_{G_M, G_C} Pr(\text{a random man is excluded}|G_M, G_C) \\ &\quad \times Pr(G_M, G_C) \\ &= \sum_{i=1}^{n} p_i\left(1-p_i+p_i^2\right)(1-p_i)^2 \\ &\quad + \sum_{i=1}^{n-1}\sum_{j=i+1}^{n} p_i p_j (p_i+p_j)(1-p_i-p_j)^2. \end{aligned} \quad (1)$$

An equivalent but simpler expression takes the form

$$Q_1 = \sum_{i=1}^{n} p_i(1-p_i)^2 - \sum_{i=1}^{n-1}\sum_{j=i+1}^{n} p_i^2 p_j^2(4-3p_i-3p_j). \quad (2)$$

Occasionally, the genotype of the mother may not be available. The formula for the PE in no-mother cases is

$$Q_2 = \sum_{i=1}^{n} p_i^2(1-p_i)^2 + \sum_{i=1}^{n-1}\sum_{j=i+1}^{n} 2p_i p_j\left(1-p_i-p_j\right)^2. \quad (3)$$

Since it is easier to exclude a man from being the child's father if the mother's genotype is available, we have $Q_1 \geq Q_2$. The analytical proof is given in the Appendix. The exclusion of paternity in no-mother cases was also discussed by Garber and Morris (1983), Lee et al. (1980), and Jamieson and Taylor (1997).

Exclusion of relatives of true father from paternity

The PE is the proportion of the male population that would be excluded as the father of a child based on the genetic information obtained. This measure is useful in evaluating the power of excluding paternity for genetic markers and so is commonly employed in the parentage testing field. However, the male population is so large that the majority of this population would be less likely to be involved in a particular paternity dispute. To assess the power of a test from another perspective, the scope of computation can be limited to a much smaller subpopulation, say, relatives of the true father. The calculation of PE under such consideration can be done by using the kinship coefficients. For two persons S_1 and S_2, the kinship coefficients (k-coefficients) are defined as:

- k_2 The probability that both alleles of S_1 and S_2 are identical by descent (ibd)
- k_1 The probability that a specific allele of S_2 is ibd to one of the alleles of S_1 and the other is not
- k_0 The probability that none of the alleles of S_1 and S_2 is ibd.

These k-coefficients (where $k_0 + 2k_1 + k_2 = 1$) have been designed for kinship analysis (see for example Wenk et al. 1996) and can be used in the formulation of the power of excluding relatives of the true father, that is to be discussed below.

Suppose R is a relative of the true father (TF). The relationship between R and TF can be expressed in terms of their kinship coefficients. For instance, if R is a brother of TF, then $k_0 = k_1 = k_2 = 0.25$. On the assumption of the mother (M) of the child (C) being unrelated to both TF and R, the child will share exactly one ibd allele with TF, and the relationship between C and R can be described by the following probabilities:

Pr (both alleles of C and R are ibd) = 0,
Pr (exactly one allele of C and one allele of R are ibd) = $k_1 + k_2$,
Pr (none of the alleles of C and R is ibd) = $k_0 + k_1$.

If C and R share no ibd allele, R can be treated as a random man unrelated to C concerning the alleles in question. Since R can be excluded only when he shares no ibd allele with C, the probability of excluding R (PER) as the father can be computed by:

$$\begin{aligned} Q_3 &= Pr(\text{R shares no ibd allele with C}) \\ &\quad \times Pr(\text{R can be excluded}|\text{R shares no ibd allele with C}) = (k_0 + k_1)Q_1. \end{aligned} \quad (4)$$

A simple relationship between the PER and the common PE is found to be held as follows: when we are concerned with the probability of the exclusion of a relative of the true father instead of a random man, the power of exclusion is reduced by a proportion equal to:

$$\begin{aligned} \frac{Q_1 - Q_3}{Q_1} &= 1 - k_0 - k_1 = k_1 + k_2 \\ &= \frac{1}{2}E(\text{number of ibd alleles of R and TF}). \end{aligned}$$

From this, it is obvious that the closer the relationship between R and TF is, the smaller the power of exclusion. This is consistent with the foregoing that it will be harder to exclude R from paternity, as R has a higher chance to inherit and share the same genetic materials with TF.

This simple relationship between the PER and the common PE holds for other situations as long as the

mother can be assumed to be unrelated to both R and TF. In particular for no mother cases, the corresponding PER is given by

$$Q_4 = (k_0 + k_1)Q_2. \quad (5)$$

It must be stressed that a change in the calculations would result if the mother bears a biological relationship with R and TF.

Results and discussion

The recent trend in the use of different technologies for parentage testing has shown that PCR-based STR typing has become extremely popular among parentage testing laboratories (Annual Report Summary for 1998 prepared by the Parentage Testing Standards Committee of the American Association of Blood Banks) and the Government Laboratory of the Hong Kong Special Administrative Region is no exception. For the problem of illegitimacy, nine STR loci (D3S1358, vWA, FGA, D5S818, D13S317, D7S820, D8S1179, D21S11, D18S51) detected by the Applied Biosystems Profiler Plus kit are normally used and if necessary, three more STR loci (TH01, TPOX, CSF1PO) detected by either the Applied Biosystems Profiler or Promega CTT kits can be done with a view to enhancing the discriminatory power of the battery of tests or obtaining a better inclusion probability for non-exclusion cases. We have evaluated the usefulness of this panel of tests by analysing the allele frequency distributions of these 12 loci based on 284 unrelated Hong Kong Chinese (Wong et al. 2001). No deviations from Hardy-Weinberg equilibrium were observed. The power of exclusion under different considerations is shown in Table 1, in which the quantities Q_3 and Q_4 have been computed based on the situation of a sibling relationship, i.e. the power of excluding an uncle of the child from paternity when the mother is available for testing and when the mother is unavailable, respectively. The sibling relationship is chosen because it is one of those that give the lowest $k_0 + k_1$ (0.5) among all possible biological relationships. Those other relationships that give the same lowest $k_0 + k_1$, i.e. 0.5, are impersonations of the true father by the grandfather or by the elder brother of the child. Q_3 and Q_4 are the 'lower bound' values for the power of exclusion for relatives. Relationships other than those three mentioned would give a higher value for the power of exclusion for the tests.

Table 1 Power of exclusion for Hong Kong Chinese, with kinship coefficients $k_0 + k_1 = 0.5$ and making no allowances for a mismatch

Locus	Probabilities of exclusion			
	Q_1	Q_2	Q_3	Q_4
D3S1358	0.4745	0.3037	0.2372	0.1519
vWA	0.6099	0.4325	0.3049	0.2162
FGA	0.7317	0.5748	0.3658	0.2874
D5S818	0.5919	0.4139	0.2960	0.2069
D13S317	0.5694	0.3905	0.2847	0.1953
D7S820	0.5481	0.3680	0.2740	0.1840
D8S1179	0.7023	0.5368	0.3512	0.2684
D21S11	0.6625	0.4915	0.3312	0.2458
D18S51	0.7315	0.5748	0.3658	0.2874
THO1	0.4496	0.2798	0.2248	0.1399
TPOX	0.3303	0.1862	0.1651	0.0931
CSF1PO	0.4992	0.3244	0.2496	0.1622
Overall (first 9 loci)	0.99988	0.99620	0.96617	0.90300
Overall (12 loci)	0.99998	0.99849	0.98357	0.93659

As can be seen in Table 1, the loci TPOX, THO1 and CSF1PO rank first, second and fourth, respectively from the bottom in terms of their PE among the 12 STR loci. The data seem to support the choice of these three loci, which are tested in a multiplex, as a supplementary system. The probabilities of excluding a random man as the true father based on each locus detected by the Profiler Plus kit range from 47% to 73% when the genotype of the mother is available. Nevertheless, when the uncle of a child claims to be the true father without the availability of the mother for testing as in an immigration application for reunion, the probabilities of exclusion become much smaller, ranging from 15% to 29%. The combined PER for the Profiler Plus system in the latter case is only 90.3%, which implies the insufficiency of this system in resolving paternity dispute for such cases. In other words, the Profiler Plus system cannot exclude 9.7% of the alleged fathers in nephew/niece-uncle cases for paternity determinations, although it performs reasonably well in the child-random man cases ($Q_2 = 0.9962$). If all the 12 loci are used, the combined PER becomes 93.66%. As the traditional PE tends to overstate the exclusion ability of a panel of tests, it is recommended to compute the PER as well for a better assessment of the effectiveness of the test battery.

For a conclusion of non-paternity, exclusions in more than one or two loci are practically required as the possibility of mutations or null alleles cannot be ignored (see for example Chakraborty et al. 1974; Kaye 1990; Chakraborty and Stivers 1996; Gunn et al. 1997; Brinkmann et al. 2001). To address this, the following formulae are available. Let P_i, $(i = 1,2,...,m)$, be the probability of exclusion based upon the ith locus. The overall probabilities of exclusion for a test battery when one and two genetic inconsistencies in the tested subjects are permitted are given by:

$$Q' = 1 - \prod_{i=1}^{m}(1 - P_i) - \sum_{i=1}^{m} P_i \prod_{\substack{j=1 \\ j \neq i}}^{m}(1 - P_j) \quad (6)$$

and

$$Q'' = Q' - \sum_{i<j} P_i P_j \prod_{\substack{k=1 \\ k \neq i, k \neq j}}^{m}(1 - P_k) \quad (7)$$

respectively.

Consequently, the effective PE/PER for a panel of tests shall be lower than the PE/PER calculated as per Eqs. 1, 3,

Table 2 Power of exclusion for Hong Kong Chinese, with kinship coefficients $k_0 + k_1 = 0.5$ and making allowances for at most one or two mismatches

Number of loci	Probabilities of exclusion			
	Q_1	Q_2	Q_3	Q_4
9[a]				
At most 1 mismatch	0.99797	0.96597	0.82627	0.64271
At most 2 mismatches	0.98439	0.86083	0.57030	0.33481
12[a]				
At most 1 mismatch	0.99957	0.98487	0.90213	0.73738
At most 2 mismatches	0.99622	0.92950	0.71846	0.45378

[a] Same as those in Table 1

4 and 5. The effective PE/PER calculated for the 9 and 12 loci as stated in Table 1 using Eqs. 6 and 7 is reported in Table 2. The PER's, i.e. Q_3 and Q_4, are not that high (highest being 0.90213) when allowances for mismatches are made.

In view of the above, it therefore seems inappropriate, at least in immigration cases for the time being, to use verbal predicates such as "practically proved" (Melvin et al. 1988) to interpret combined parentage indexes obtainable from currently used parentage tests.

Appendix. By subtracting Eq. 3 from Eq. 1, we have:

$$
\begin{aligned}
Q_1 - Q_2 &= \sum_{i=1}^{n} p_i (1-p_i)^2 (1 - 2p_i + p_i^2) \\
&\quad - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} p_i p_j (2 - p_i - p_j)(1 - p_i - p_j)^2 \\
&= \sum_{i=1}^{n} p_i (1-p_i)^4 - \sum_{i=1}^{n} \sum_{j \neq i} p_i p_j (1-p_i)(1 - p_i - p_j)^2 \\
&\geq \sum_{i=1}^{n} p_i (1-p_i)^4 - \sum_{i=1}^{n} p_i (1-p_i)^3 \sum_{j \neq i} p_j = 0
\end{aligned}
$$

which establishes the inequality.

Acknowledgements The authors are grateful to the Editor and a referee who suggested a simple proof of our mathematical result and to another referee for comments on mutation which improved the presentation of the paper. The authors thank Dr. D.G. Clarke, Government Chemist of the Government Laboratory of the Hong Kong Special Administrative Region, and Mr. S.C. Leung, Assistant Government Chemist, for their administrative support and proof-reading the draft. The first author was partly supported by the Hong Kong RGC Competitive Earmarked Research Grant HKU 7136/97H.

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