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Problems and Pitfalls in Blood Grouping Tests for Disputed Parentage

III. Chances of Proving Non-Paternity by Blood Grouping Tests when the Putative Father is Dead [1]

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1. Introduction

Recently an unusual problem of disputed paternity was posed to the writer. The parents of a man who had recently died were concerned about their son's two children. They wished to adopt the older of the two children, because they considered the mother, their daughter-in-law, to be irresponsible, and mentally unfit to raise the child. However, they wanted no part of the younger grandchild, because they believed he was not the natural child of their son. The attorney for the grandparents therefore consulted the writer to inquire whether this problem could be solved by blood tests, because he had been told that the problem could not be solved. The writer assured him that the blood tests could solve a certain proportion of problems of this nature. The purpose of the present paper is to give the chances of solving such problems, and to compare the chances with those when the putative father as well as the mother are available for testing.

2. Simple Mendelian Dominant

This is the simplest case, namely, a blood group system for which only a single kind of antiserum is available for testing, e. g. the Kell system, in which for practical purposes tests are ordinarily restricted to those with anti-**K** serum alone, thus defining only two blood types. Let us consider the general case, in which the antiserum may

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be designated as anti-**D**, and the two blood types as D+ and D —, respectively. Heredity is ordinarily by a single pair of allelic genes, which may be designated as *D* and *d*, respectively, where *D* determines the presence of agglutinogen D, while gene *d* is an amorph. Thus, there are three genotypes corresponding to the two phenotypes as follows: phenotype D +, genotypes *DD* and *Dd*; and phenotype D —, genotype *dd*. The only instance in which useful results are possible in a case of disputed paternity is one where both the paternal grandparents, i. e. the parents of the deceased father, are D —. Then, paternity will be excluded if it is found that the mother is also D — while the child is D +.

If the frequencies of the two phenotypes in the general population be taken as follows: D + = D and D — = R, and at the same time the frequencies of the two allelic genes be taken as *D* = p and *d* = q, then obviously, $R = q^2$ and $D = p^2 + 2pq$. Moreover, the chances of excluding paternity, P, is then given by the formula:

$$P = R^3 p. \qquad [1]$$

For the Kell blood types, which in white populations have the approximate distribution: K + = 0.10 and K — = 0.90, so that q = 0.95 and p = 0.05,

$$P = (0.90)^3 (0.05) = 0.036 \text{ or } 3.6\ \%.$$

The maximum chances of proving non-paternity in such a two-allele system is determined as follows:

$$P = R^3 p = pq^6 = q^6 - q^7. \qquad [2]$$

Therefore, $\frac{dP}{dq} = 6q^5 - 7q^6$.

Since P assumes its maximum value when $\frac{dP}{dq} = 0$, we set $6q^5 - 7q^6 = 0$

So that q = 6/7 and p = 1/7.

Thus, the distribution of types that gives the maximum chances of excluding paternity is D + = 0.265 and D — = 0.735, and the maximum chances of exclusion are:

$$P = (0.735)^3\ 0.143 = 0.055 \text{ or } 5.5\ \%.$$

This may be compared with the chances of proving non-paternity when the falsely accused putative father is alive and available for testing (Wiener et al, 1930). In that case, $P = R^2p$, which for the Kell types becomes $P = (0.90)^2\ 0.05 = 0.0405$ or 4.05 %.

The maximum value of P in this case occurs when q = 4/5 and p = 1/5, i. e., when R = 0.64 and D = 0.36. Thus, the maximum value of $P = (0.64)^2\ 0.2 = 0.819$ or 8.19 %.

It is of interest to note, therefore, that in the simplest case of a blood group system with only a single kind of antiserum the chances of excluding a man who is falsely suspected of being the father of a child is only moderately reduced if the putative father is dead, provided that both his parents are alive and available for testing.

3. Two Codominant Genes

The three M-N blood types, which are inherited by a pair of codominant alleles, *M* and *N*, provide an example of the general problem of excluding paternity by tests for a blood group system inherited by two codominant allelic genes. The instances in which a deceased man, falsely accused of the paternity of a child, can be excluded are set out in Tab. I. Thus, if the frequencies of the three M-N types be indicated

Tab. I. Chances of proving non-paternity by M-N tests on mother, child and putative father

Paternal grandparents	Mother	Child	Frequency
M×M	M	MN	$\overline{M}^2m^2n$
	MN	N	$\overline{M}^2mn^2$
	N	N	$\overline{M}^2n^3$
	Above 3 possibilities combined		$\overline{M}^2n(1-mn)$
N×N	M	M	$\overline{N}^2m^3$
	MN	M	$\overline{N}^2m^2n$
	N	MN	$\overline{N}^2mn^2$
	Above 3 possibilities combined		$\overline{N}^2m(1-mn)$
M×M	Not tested	N	$\overline{M}^2\cdot N$
N×N	Not tested	M	$\overline{M}\cdot N^2$

by a bar above the appropriate phenotype symbols as follows: $\overline{M}$, $\overline{N}$ and $\overline{MN}$, and the frequencies of the two alleles as $m = M$ and $n = N$, then the total chances of excluding paternity are:

$$P = \overline{M}^2n(1 - mn) + \overline{N}^2m(1 - mn) \quad [2]$$

$$\text{Thus, } P = m^4n(1 - mn) + mn^4(1 - mn) = mn(1 - mn)(1 - 3mn) \quad [3]$$

These chances attain their maximum value when $m = n = \frac{1}{2}$, which is the approximate situation for most Caucasian and Negro populations. Thus, the maximum chances of excluding paternity are 3/64 or 4.7 %. In this case, therefore, the chances of exclusion are one fourth the maximum chances when the putative father is alive and available for testing, the latter being 18.75% (Wiener et al, 1930; Wiener, 1943).

If only the putative father's parents, and not the mother of the child, are available for testing, the chances of excluding paternity become (cf Tab. I):

$$P = \overline{M}^2\cdot\overline{N} + \overline{M}\cdot\overline{N}^2 = \overline{M}\cdot\overline{N}\ (1 - \overline{MN}) \quad [4]$$

$$\text{or } P = m^4n^2 + m^2n^4 = m^2n^2(m^2 + n^2) \quad [5]$$

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Since this attains its maximum value when $m = n = \frac{1}{2}$, the maximum chances of proving non-paternity when neither the putative father nor the mother are available, but the putative father's parents are available for testing, are 1/32 or about 3.125 %. The same applies to the chances of proving non-maternity when the putative mother is not alive, but the maternal grandparents are available, as might occur in immigration or kidnapping cases.

These chances again are one-fourth the chances of excluding parentage (paternity or maternity) when the putative parents are alive but only the parent in question is available for testing, e. g. where a man suspects that he is not the father of his wife's child and wishes to have blood tests carried out on him and the child without his wife's knowledge. In such cases $P = 2 \cdot \overline{M} \cdot \overline{N} = 2m^2n^2$, which has the maximum value of 0.125 or 12.5%.

4. Triple Allelic Genes: Two Codominant Genes and the Third an Amorph

The A-B-O blood groups provide the outstanding example of a system of blood groups where inheritance is by triple allelic genes, two of which are codominant and the third an amorph.

As is customary, let the frequencies of the four blood groups be designated by the appropriate phenotype symbol with a bar above it, namely, $\overline{O}$, $\overline{A}$, $\overline{B}$ and $\overline{AB}$, respectively, while the frequencies of the three alleles, *A*, *B* and *O* are represented by the letters p, q and r, respectively. Then the formulae for the chances of excluding paternity by A-B-O blood tests on the mother, child and both paternal grandparents are derived as shown in Tab. II. The total chances of disproving paternity are obtained by taking the sum of the chances given in the last column of Tab. II.

Tab. II. Exclusion of paternity based on tests for the A-B-O blood groups on mother, child and both parents of the putative father

Paternal grandparents	Mother	Child	Frequency
O×O O×A A×O A×A	O A	B B or AB	$(\overline{O}+\overline{A})^3q$
O×O O×B B×O B×B	O B	A A or AB	$(\overline{O}+\overline{B})^3p$
O×O	AB	AB	$\frac{1}{2}\overline{O}^2\overline{AB}(1-r)$
AB×AB	—	O	$\overline{AB}^2 \cdot \overline{O}$
O×O	Not tested	AB	$\overline{O}^2 \cdot \overline{AB}$
AB×AB	Not tested	O	$\overline{AB}^2 \cdot \overline{O}$

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Thus, $P = (\overline{O} + \overline{A})^3 q + (\overline{O} + \overline{B})^3 p + \frac{1}{2}\overline{O}^2 \cdot \overline{AB}\ (1 - r) + \overline{AB}^2 \cdot \overline{O}$ [6]

For example, if we consider a population where p = 0.20, q = 0.20 and r = 0.60, such that $\overline{O} = 0.36$, $\overline{A} = 0.28$, $\overline{B} = 0.28$ and $\overline{AB} = 0.08$, then P = 10.9%, with which may be compared the chances when tests are done on the putative father, mother and child, which are 19.8 %. As another example, in a population where p = 0.15, q = 0.15 and r = 0.70, such that $\overline{O} = 0.49$, $\overline{A} = 0.2325$, $\overline{B} = 0.2325$ and $\overline{AB} = 0.045$, then P = 11.5 %, which compares even more favorably with the chances of exclusion when the falsely accused man is also tested — in this case, 18.5 %.

The chances of exclusion of parentage are greater if tests are carried out also for the A_1-A_2 subgroups, but the increase in the chances is relatively small. The instances that permit exclusion are those where neither of the paternal grandparents nor the mother belongs to subgroup A_1 or subgroup A_1B, while the child is of subgroup A_1 or A_1B, and also the case where both paternal grandparents are of subgroup A_1B and the grandchild of subgroup A_2. In the latter case, tests on the mother's blood are neither necessary nor relevant. However, in view of the low frequency of subgroup A_1B, the latter case would be rare, and in the nature of a curiosity or museum piece.

5. Three Codominant Allelic Genes

Tests for the Rh-Hr blood types, as ordinarily carried out for medicolegal problems of disputed parentage, include the use of sera of specificities anti-**Rh_0**, anti-**rh′**, anti-**rh″**, anti-**rh^{w_1}**, anti-**hr′**, anti-**hr″** and, where indicated, also with anti-**hr.** The resulting scheme of phenotypes and genotypes involves at least ten alleles, and is too complex for simple analysis for the calculation of the chances of excluding paternity. Let us consider instead, therefore, the results only of tests with the three antisera, anti-**rh′**, anti-**rh″**, and anti-**hr**, which comprise the bulk of the chances of excluding paternity by the Rh-Hr blood tests. Such a limited set of tests gives rise to a scheme of six phenotypes, inherited by triple codominant allelic genes.

Let us, therefore, consider the general case of triple codominant alleles, *R*, *S* and *T*, giving rise to a simple scheme of six phenotypes with six corresponding genotypes. Let the frequencies of the alleles *R*, *S* and *T* in the general population be r, s and t, respectively, and let the frequencies of the six phenotypes be represented by the appropriate phenotype symbols with a bar above the symbol. To simplify the calculations further, we shall consider chiefly the special case of that distribution of phenotypes (and genotypes) which gives the maximal chances of excluding paternity, namely, when r = s = t = 1/3, so that $\overline{R} = \overline{S} = \overline{T} = 1/9$, and $\overline{RS} = \overline{RT} = \overline{ST} = 2/9$.

For orientation, in Tab. III are set forth the calculations for the chances of proving non-paternity when the falsely accused man is alive and available for testing together with the mother and child (cf Wiener, 1968). As shown in Tab. III, when the distribution of the types in the general population is ideal, the chances that paternity will be excluded are 10/27, so that as many as 37.0% of the cases can be solved

Tab. III. Chances of proving non-paternity with a system determined by triple allelic codominant genes, by tests on mother, child and putative father

Putative father	Mother	Child	Frequency of exclusion of paternity: Formula	Frequency of exclusion of paternity: Value, assuming ideal distribution of types*
R	—	S, T or ST	$\overline{R}(\overline{S}+\overline{T}+\overline{ST})$	4/81
S	—	R, T or RT	$\overline{S}(\overline{R}+\overline{T}+\overline{RT})$	4/81
T	—	R, S or RS	$\overline{T}(\overline{R}+\overline{S}+\overline{RS})$	4/81
RS	—	T	$\overline{RS}\cdot\overline{T}$	2/81
RT	—	S	$\overline{RT}\cdot\overline{S}$	2/81
ST	—	R	$\overline{ST}\cdot\overline{R}$	2/81
R	R	RS or RT	$\overline{R}^2(1-r)$	2/243
	RS	RT	$2\overline{R}rst$	2/243
	RT	RS		
S	S	RS or ST	$\overline{S}^2(1-s)$	2/243
	RS	ST	$2\overline{S}rst$	2/243
	ST	RS		
T	T	RT or ST	$\overline{T}^2(1-t)$	2/243
	RT	ST	$2\overline{T}rst$	2/243
	ST	RT		
RS	R	RT	$\overline{RS}(\overline{R}+\overline{S}+\overline{RS})t$	8/243
	S	ST		
	RS	RT or ST		
RT	R	RS	$\overline{RT}(\overline{R}+\overline{T}+\overline{RT})s$	8/243
	T	ST		
	RT	RS or ST		
ST	S	RS	$\overline{ST}(\overline{S}+\overline{T}+\overline{ST})r$	8/243
	T	RT		
	ST	RS or RT		

* Obtained by taking $r = s = t = 1/3$, $\overline{R} = \overline{S} = \overline{T} = 1/9$ and $\overline{RS} = \overline{RT} = \overline{ST} = 2/9$.

by such tests. The analogous problem involving a putative father who is dead, but whose parents are available for testing is set out in Tab. IV. As shown in Tab. IV the maximum chances of excluding paternity under such conditions are reduced to 38/243 or 15.6 %, so that about two-fifths as many cases can be solved under such circumstances. In tests for the Rh-Hr blood types where antisera of seven different specificities are used instead of only three, one might expect the chances of exclusion to be higher, except that the distribution of the types in the general population are in general different from the optimal distribution for exclusion. Bearing this in mind, the figure 15.6 % may be taken as a fair estimate of the chances of excluding paternity by the Rh-Hr tests where the falsely accused man is dead, but his parents are available for testing.

Tab. IV. Chances of proving non-paternity with a system determined by triple allelic codominant genes, by tests on the mother, child and both paternal grandparents

Paternal grandparents	Mother	Child	Frequency of exclusion of paternity	
			Formula	Value, assuming ideal distribution of types *
R×R	—	S, T or ST	$\overline{R}^2(\overline{S}+\overline{T}+\overline{ST})$	4/729
S×S	—	R, T or RT	$\overline{S}^2(\overline{R}+\overline{T}+\overline{RT})$	4/729
T×T	—	R, S or RS	$\overline{T}^2(\overline{R}+\overline{S}+\overline{RS})$	4/729
R×S	—	T	$2\overline{R}\cdot\overline{S}\cdot\overline{T}$	2/729
R×T	—	S	$2\overline{R}\cdot\overline{S}\cdot\overline{T}$	2/729
S×T	—	R	$2\overline{R}\cdot\overline{S}\cdot\overline{T}$	2/729
R×RS	—	T	$2\overline{R}\cdot\overline{RS}\cdot\overline{T}$	4/729
RS×RS	—	T	$\overline{RS}^2\cdot\overline{T}$	4/729
S×RS	—	T	$2\overline{S}\cdot\overline{RS}\cdot\overline{T}$	4/729
R×RT	—	S	$2\overline{R}\cdot\overline{RT}\cdot\overline{S}$	4/729
RT×RT	—	S	$\overline{RT}^2\cdot\overline{S}$	4/729
T×RT	—	S	$2\overline{T}\cdot\overline{RT}\cdot\overline{S}$	4/729
S×ST	—	R	$2\overline{S}\cdot\overline{ST}\cdot\overline{R}$	4/729
ST×ST	—	R	$\overline{ST}^2\cdot\overline{R}$	4/729
T×ST	—	R	$2\overline{T}\cdot\overline{ST}\cdot\overline{R}$	4/729
R×R	R	RS or RT	$\overline{R}^3(1-r)$	2/2187
	RS	RT	$2\overline{R}^2rst$	2/2187
	RT	RS		
S×S	S	RS or ST	$\overline{S}^3(1-s)$	2/2187
	RS	ST	$2\overline{S}^2rst$	2/2187
	ST	RS		
T×T	T	RT or ST	$\overline{T}^3(1-t)$	2/2187
	RT	ST	$2\overline{T}^2rst$	2/2187
	ST	RT		
R×S	R	RT	$2\overline{R}\cdot\overline{S}(\overline{R}+\overline{S}+\overline{RS})t$	8/2187
	S	ST		
	RS	RT or ST		
R×T	R	RS	$2\overline{R}\cdot\overline{T}(\overline{R}+\overline{T}+\overline{RT})s$	8/2187
	T	ST		
	RT	RS or ST		
S×T	S	RS	$2\overline{S}\cdot\overline{T}(\overline{S}+\overline{T}+\overline{ST})r$	8/2187
	T	RT		
	ST	RS or RT		
R×RS	R	RT	$2\overline{R}\cdot\overline{RS}(\overline{R}+\overline{S}+\overline{RS})r$	16/2187
RS×RS	S	ST	$\overline{RS}^2(\overline{R}+\overline{S}+\overline{RS})t$	16/2187
S×RS	RS	RT or ST	$2\overline{S}\cdot\overline{RS}(\overline{R}+\overline{S}+\overline{RS})t$	16/2187
R×RT	R	RS	$2\overline{R}\cdot\overline{RT}(\overline{R}+\overline{T}+\overline{RT})s$	16/2187
RT×RT	T	ST	$\overline{RT}^2(\overline{R}+\overline{T}+\overline{RT})s$	16/2187
T×RT	RT	RS or ST	$2\overline{T}\cdot\overline{RT}(\overline{R}+\overline{T}+\overline{ST})s$	16/2187
S×ST	S	RS	$2\overline{S}\cdot\overline{ST}(\overline{S}+\overline{T}+\overline{ST})r$	16/2187
ST×ST	T	RT	$\overline{ST}^2(\overline{S}+\overline{T}+\overline{ST})r$	16/2187
T×ST	ST	RS or RT	$2\overline{T}\cdot\overline{ST}(\overline{S}+\overline{T}+\overline{ST})r$	16/2187

* Obtained by taking $r=s=t=1/3$; $\overline{R}=\overline{S}=\overline{T}=1/9$ and $\overline{RS}=\overline{RT}=\overline{ST}=2/9$.

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6. Combined Chances of Excluding Paternity

To calculate the chances of excluding paternity by the combined use of tests for A-B-O, M-N, Rh-Hr and Kell, one uses the following formula:

$$P = 1 - (1 - P_1)\ (1 - P_2)\ (1 - P_3)\ (1 - P_4)...(1 - P_n) \qquad [6]$$

where P_1 are the chances of exclusion by the first blood group system, P_2 the chances for the second independently inherited blood group system, etc.

Substituting the estimates that have been derived above, one obtains

$$P = 1 - (1 - 0.156)(1 - 0.047)(1 - 0.112)(1 - 0.036) = 0.311 \text{ or } 31.1\%.$$

These combined chances of excluding paternity prove to be substantial, so that it is very much worth while to undertake blood grouping tests for the establishment of non-paternity, even though the falsely accused man is dead, provided that both parents are available for testing.

Summary

General formulae for the chances of excluding paternity by blood grouping tests have been derived for the case where the falsely accused man is dead, but both his parents are available for testing. With the aid of the formulae, the chances of excluding paternity are shown to be about 31.1 %, when tests are carried out for the A-B-O blood groups and subgroups, the M-N types, the Rh-Hr types and the Kell factor. These combined chances are about half the chances of exclusion when the putative father is alive and can be tested, so that it is very much worth while to carry out blood grouping tests in cases of this nature.

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RIASSUNTO

Le formule generali per il calcolo della probabilità di esclusione di paternità sulla base dei gruppi sanguigni sono state applicate al caso in cui l'uomo accusato sia morto, ma ne siano disponibili ambedue i genitori. Mediante tali formule, le probabilità di escludere la paternità si configurano intorno al 31.1%, sulla base di tests eseguiti per i gruppi e sottogruppi ABO, MN, Rh-Hr e Kell. Queste probabilità combinate rappresentano circa la metà di quelle di esclusione quando il padre putativo sia vivo e possa essere sottoposto ai tests, cosicché la ricerca gruppale in casi di questo genere presenta una notevole utilità.

RÉSUMÉ

Les formules générales pour calculer la probabilité d'exclusion de paternité, sur la base des groupes sanguins, ont été appliquées au cas de la mort de l'homme accusé, ses parents étant encore disponibles. Par ces formules, les probabilités d'exclusion de paternité s'élèvent à 31.1% environ, sur la base des tests pour les groupes ABO, MN, Rh-Hr et Kell. Ces probabilités combinées représentent la moitié environ de celles d'exclusion, dans le cas où le père putatif est vivant et peut être soumis aux tests. La recherche des groupes sanguins dans ces cas se présente, par conséquent, très utile.

ZUSAMMENFASSUNG

Für den Fall, daß ein der Vaterschaft angeklagter Mann verstorben, aber seine beiden Eltern zugänglich seien, wurden die allgemeinen Formelen zur Wahrscheinlichkeitsrechnung des Vaterschaftsausschlusses auf Grund der Blutgruppen angewandt. Mit diesen Formeln betrug die Wahrscheinlichkeit, die Vaterschaft auf Grund der Tests mit den Blutgruppen und Untergruppen ABO, MN, Rh-Hr und Kell auszuschließen, ungefähr 31.1% Diese kombinierten Wahrscheinlichkeiten für die Vaterschaftsausschließung entsprechen ungefähr der Hälfte derjenigen, die sich ergeben, wenn der vermutliche Vater lebt und getestet werden kann, so daß sich also die Blutgruppenuntersuchung in solchen Fällen als äußerst nützlich erweisen.

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