Paternity Exclusion and Probability of Paternity

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ABSTRACT

The principles of paternity exclusion and current systems of paternity testing are reviewed. Recent advances, especially the utilization of human leukocyte antigens (HLA), have greatly improved the likelihood of exclusion of the innocent accused male. Failure to exclude an alleged father through multiple test systems increases the suspicion that he is the biological father. This can be expressed mathematically as the Probability of Paternity, for which the principle of calculation is explained. Legal obligations of the laboratory are briefly explored.

Introduction

The field of paternity testing is currently in rapid evolution from both the scientific and the legal point of view. The courts have granted increasing recognition and rights to children born out of wedlock and thus raised the importance of establishing paternity. Federal legislation passed in 1975 offers incentives to states to identify the reluctant father so that he can be held responsible for support, thus reducing the cost to the taxpayer of unsupported children. Blood tests as described have an obvious role to play in this effort. Documentation of parentage may be important in other situations, as when the possibility is raised of infants being switched in the nursery. In a divorce action, a father may seek to establish that one or more of the children are not his own. Parentage may be important in immigration proceedings.

Paternity testing is based on classical Mendelian patterns of inheritance. Individual genes disclose themselves by the presence of identifiable somatic end-products. For purposes of paternity testing, the genetic system under study must possess: (1) a single and unequivocal pattern of inheritance; (2) accurate classification of different phenotypes by reliable techniques; (3) a relatively high frequency of each of the common alleles; and (4) absence of environmental factors, age, interaction with other genes, or other variables in the expression of the trait.

It is usually assumed that the mother is the true mother of the child. Mutations have been shown to be vanishingly rare in these systems. Unusual circumstances such as suppressor genes, low-frequency
silent alleles, crossover, etc., are identifiable problems which must be considered and excluded when appropriate.

An alleged father may be excluded when his genetic makeup is inconsistent with that of the mother and child, as deduced from a study of the respective phenotypes. There are two categories of paternal exclusion reflecting descending degrees of certainty (figure 1).

(1) First Order Exclusion: A child possesses a character which is not found in either parent. Very rarely false first order exclusions may be caused by suppressor genes, by lack of an earlier enzyme in the biochemical pathway, by chimerism or by mutation.

(2) Second Order Exclusion: An alleged father possesses characters which he must pass on to all his children but which are absent from this child. Examples include homozygous traits, or AB blood type, not expressed in the child. Second order exclusions, since they depend on negative rather than on positive findings in the child, are less firm and usually require confirmation. False second order exclusions may be caused by silent or amorphic genes, unidentified alleles, and by the causes listed for false first order exclusions.

Four kinds of genetic markers have been put to practical use in paternity testing. These are set apart by the techniques used to identify the gene products. The systems also vary in their efficacy at excluding the innocent alleged father (table I). Efforts toward exclusion from paternity become cumulatively more effective as more tests are performed. This is expressed in the following formula, (where C.E. is the chance of exclusion by each test):

\[
\text{Cumulative Chance of Exclusion} = 1 - (1 - C.E._1)(1 - C.E._2)(1 - C.E._3) \ldots \text{etc.}
\]

The formula is applicable for accumulating the efficacy of tests within a group or for using several groups together. The exact figures can be derived from the gene frequencies and therefore vary somewhat between racial groups.

**Blood Groups**

Historically the blood groups were the first human antigenic systems to be explored in detail. This was begun by Landsteiner in 1900 with the discovery of the ABO system; ABO blood typing was applied to paternity exclusion in the United States in the 1930's. Other erythrocyte antigens have been introduced as knowledge has accumulated. The MNSs system is the most useful with about 30 percent chance of exclusion. The Rh system is slightly less useful while the ABO system offers approximately 20 percent exclusion. The ABO system is flawed by the excessively high frequency of the “A” allele(s) and by the high frequency amorph allele “O.”

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**Figure 1. First Order Paternity Exclusion.** The child possesses a character which is not found in either parent. In this example the biological father of this child contributed an A gene.

**Figure 2. Second Order Paternity Exclusion:** All the children of this father must carry either an A or a B gene from the father. In this example the father cannot have a type O child.
While the blood group antigens are the traditional bulwark of paternity testing and are well established in the courts, application requires a detailed knowledge of the numerous silent alleles, occasional suppressor genes and unusual combinations which may lead to false exclusions. Other systems and techniques have been explored in order to extend the principle and to increase the power of exclusion.

Serum Proteins and Erythrocyte Enzymes

Genetically determined polymorphisms are readily detected in many proteins, some of which are easily accessible in serum and in erythrocytes. These groups have in common that they are separable by electrophoresis. Serum proteins which are in use include the following: haptoglobin, group specific component, transferrin, and ceruloplasmin. Erythrocyte enzymes include: phosphoglucomutase, adenylate kinase, and 6-phosphogluconate dehydrogenase. The most effective single serum protein system is Gm. The Gm factors are expressed as peptide variations of the heavy chain portion of the IgG molecule. Special techniques are required to separate these factors.

HLA

The HLA system, (human leukocyte antigen system, A for “the first”), has been developed primarily to facilitate organ transplantation. Four loci A, B, C, and D are known, all residing on the short arm of chromosome 6. The loci are closely associated and are usually inherited together. Crossing over does occur and is measured at one percent or less between loci A and B. Genes at all four loci are fully expressed or “co-dominant.” The genes at loci A, B, and C are expressed as surface antigens on all nucleated cells of the body. These antigens are detected by observing the cytotoxic effect of the corresponding specific antibody on lymphocytes in culture media. Complement is fixed in this reaction. Thus, A, B, and C antigens are said to be serologically detectable (SD), and their genes are serologically defined. D-locus antigens must be detected by the interaction between test and “responder” lymphocytes in the mixed lymphocyte culture (MLC) technique.

Human leukocyte antigen groups A and B have properties which make them very attractive for paternity testing: (1) both loci are very polymorphic and include many alleles of low frequency. There are 16 alleles at locus A and 21 at locus B. (2) It can be shown statistically that the alleles at loci A and B are almost fully explored; thus, the chance of undetected alleles in the white population is 0.02 for locus A and 0.03 for locus B. (3) Present techniques and available antisera permit practical identification of these antigens.

Each person inherits two genes at the A locus and two at the B locus. Most people are heterozygous at both loci because of the numerous low-frequency alleles available at each. The presence of four antigens is called a “full house.” If an
individual is homozygous at one locus there will be only three antigens expressed, and double homozygosity would lead to a two-antigen phenotype. Alternatively, a reduced number of antigens may be due to silent previously unknown alleles, or to the lack of the appropriate reagent antibody. Lack of a “full house” of antigens complicates the interpretation by introducing unknowns. The power of the results is thereby reduced but not invalidated.

A and B alleles on the same chromosome will be inherited together, (barring the one percent or less crossover possibility). The linked A and B genes together constitute a haplotype of which a child receives one from each parent. Haplotypes have a high degree of uniqueness since the haplotype frequency is approximately equal to the product of the frequency of the A- and B-locus genes. Thus, dealing in haplotypes raises further the power of paternity exclusion or probability. However, haplotypes cannot be determined from data from one individual, but can be (1) deduced from family studies or (2) hypothesized for a paternity study trio. This is similar to the derivation of the CeDEe genotype from phenotypic information. Probability tables are applied to infer the probable genotype of the individual; family studies may be used to provide certainty where necessary.

Some of the problems associated with HLA paternity testing include: (1) frequency tables for HLA genotypes and phenotypes are not fully complete, especially for non-Caucasians. These data are rapidly being developed. (2) Reference antisera are in short supply and have not been available for complete phenotyping in many centers. Reagent antisera have been developed locally and regionally; many of these sera have been polyspecific. However, excellent monospecific antisera are rapidly becoming available. (3) Certain HLA groups have predictable cross-reactivity even with monospecific typing sera. However, experienced workers can attain correct phenotype identification with certainty. In fact, phenotypic expression of genes is far more straightforward in the HLA antigens than in the blood groups.

Many laboratories have turned to HLA as their first line of attack in paternity testing because of its efficiency. The statistics support such a choice. Some HLA typing centers, originally established to support organ transplantation services, have extended their operations to include paternity testing. Such centers will usually reinforce the HLA results with blood group antigen studies where indicated; some centers may offer additional protein testing.

The cost of paternity tests are usually borne by the accused “alleged father.” The cost is definitely higher for a greater probability of exclusion. A minimal effort with common blood bank sera will be least expensive but may well not exclude the innocent male. More elaborate tests beginning with HLA will cost hundreds of dollars but may be a good investment for the accused party who is convinced of his innocence and who is determined to avoid years of support payments.

**Probability of Paternity**

It is a matter of pure logic that genetic studies can never prove that an individual man is the father of a particular child. It is always possible that another man, as yet unidentified, might be able to contribute the necessary genes. Early workers in paternity testing, armed only with the blood groups, offered only modest hope of excluding the innocent father. There was little interest in calculating a positive probability of paternity since the probability would have to be reasonably high to be of any use. The more advanced techniques have made paternity exclusion much more effective for the innocent and, at the same time, have pushed non-exclu-
EXCLUSION AND PROBABILITY OF PATERNITY

The significance of non-exclusion can be expressed numerically as the Probability of Paternity. This is based on the principle that, for each genetic system studied, one can determine the Obligatory Gene which must have been provided by the biological father. This is deduced by comparing the genetic makeup of the mother and child. Where the phenotypes permit alternative genetic makeups all possibilities are taken into account. Next, the Alleged Father’s chance of producing such a gene is listed, based on his known phenotype. The chance that a Random Male might produce the Obligatory Gene is listed in parallel for each system studied; this is simply the gene frequency in the population. The probability of the Alleged Father producing the necessary sperm is obtained by multiplying the chances for each individual gene. This product is called the X-value, while the corresponding value for the Random Male is the Y-value. The relative chance of paternity is thus X/Y, which is called the Paternity Index. It can be considered to be an expression of how much more likely is the Alleged Father to be the true father than is a Random Male. A slightly different form of expression as percentage is the Probability of Paternity = \( \frac{X}{X + Y} \times 100 \). In table II are listed the VerbalPredicates in which these values may be expressed.

While Probability of Paternity may never constitute a proof, it is useful information which may be considered by judge or jury along with other evidence and testimony.

**Legal**

Performance of paternity testing invariably implies legal as well as scientific responsibilities. The identity of the parties submitting blood samples must be firmly established at the time of venipuncture.

| Likelihood of Paternity | Paternity Index | Probability of Paternity (%)
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Practically proved</td>
<td>399 to 1</td>
<td>99.8 - 99.9</td>
</tr>
<tr>
<td>Extremely likely</td>
<td>95 to 1</td>
<td>99.0 - 99.7</td>
</tr>
<tr>
<td>Very likely</td>
<td>19 to 1</td>
<td>95.0 - 98.9</td>
</tr>
<tr>
<td>Likely</td>
<td>9 to 1</td>
<td>90.0 - 94.9</td>
</tr>
<tr>
<td>Certain hint</td>
<td>4 to 1</td>
<td>80.0 - 89.9</td>
</tr>
<tr>
<td>Not useful</td>
<td>&lt; 4 to 1</td>
<td>Less than 80</td>
</tr>
</tbody>
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Documentation with Polaroid photographs, finger and infant heel prints may be required. At this time the parties may be required to sign releases. They should be informed that the results and interpretation of the tests, while confidential, will be sent to the court and to the lawyers of both parties. Absolute chain of custody of the specimens must be maintained. To this end the same technologists may perform the venipunctures and the tests. Tests must be performed in duplicate by technologists working independently. Reagents must be properly controlled. Working records must be kept in orderly fashion and in a form presentable in court should the challenge arise.

Finally, the Laboratory Director must be willing to confirm the court of the validity of the procedures being used as well of the particular results at hand. Frequently it is his task to inform the court about new advances in the field before they have become embedded in the law. Thus 14 states now have laws or state court decisions which recognize HLA testing as valid evidence in paternity testing. In the remaining states the decision rests on judicial discretion which may very well depend on the testimony of the laboratory director.

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References


