Genetic Marker Analysis in Cases of Disputed Paternity When the Alleged Father is Deceased

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ABSTRACT

Data are presented to demonstrate the utility of genetic marker analysis in cases of disputed paternity in which the alleged father is deceased prior to testing. The first case involves the histocompatibility (HLA) typing of the alleged father's parents and presumed mother and child, in which the deceased alleged father (AF) was not excluded from paternity. The second case involves a similar situation. However, the deceased AF was not clearly excluded from paternity in the HLA system owing to the inability to rule out gene suppression or deletion, but the deceased AF was excluded following red blood cell antigen typing of the man's parents. These two cases demonstrate the utility of genetic marker analysis in cases of disputed paternity in which the AF is deceased. This same type of analysis can be applied to cases in which the presumed mother is either deceased or missing and custody of the child is being sought by the alleged father.

Introduction

In the majority of states, utilization of histocompatibility testing (HLA) is considered admissible evidence in cases involving disputed or questionable paternity. Various modalities of testing, including red blood cell (RBC) antigen typing, HLA typing, red cell enzyme and serum protein electrophoresis, can be performed with respect to paternity cases. Of the more than routinely performed laboratory tests, HLA typing alone provides the most powerful exclusionary system in that 82 to 93 out of 100 innocent alleged fathers can be excluded...
by this system. However, by combining HLA and RBC typing, along with RBC enzyme and serum protein electrophoresis, 95 to 99 percent of innocent alleged fathers can be excluded from paternity. With the advent of desoxyribonucleic acid (DNA) fingerprinting as applied to parentage testing, it is possible to attain exclusion probabilities >99 percent depending upon the number and types of DNA probes used. However, at this time it is not cost effective for most paternity testing laboratories to use this assay system solely. Currently, our laboratory uses the DNA fingerprinting in cases when a single second class exclusion is obtained by the traditional testing procedures, or when the probability of paternity (PP) is very low following RBC and HLA typing and electrophoresis.

The expected normal protocol for paternity testing would include the drawing of blood samples from the presumed mother, child and alleged father (AF), and, following prescribed analysis, the testing laboratory would provide information regarding the exclusion or inclusion of paternity.

Considering the enhanced exclusionary capabilities of HLA typing because of its highly polymorphic nature, this system can be used in unusual cases of disputed paternity. Spieser et al used HLA typing to exclude a deceased AF by testing the presumed mother and child, and parents of the AF. During the past three years, 20 cases of disputed paternity, in which the AF was deceased prior to testing, have been analyzed. Generally, the reasons for this unusual form of paternity case reflect either the child’s mother attempting to procure survivor rights, i.e., Social Security benefits owing to the death of the AF, or the potential for the child receiving inheritance rights to the alleged father’s estate. In this paper, two of the 20 cases are presented in which the paternity testing was performed by the authors when the AF was deceased prior to paternity testing.

Materials and Methods

Subjects

In cases #1 and #2, the persons tested included the presumed mother, child, and the alleged paternal grandparents.

HLA Typing

The microcytotoxicity assay described by Kissmeyer-Nielsen and Dick was followed for the HLA typing using Terasaki HLA Trays and additional trays after the blood was drawn from the respective parties.

Red Blood Cell Antigen Typing

The RBC from the persons tested in Case #2 were examined for the RBC antigens of the following blood group systems: ABO (A,A,1,B), Rh (D,C,c,E,e), MNSs, Kell (K,k), Duffy (Fya,Fyb), and Kidd (Jka,Jkb), using appropriate antisera. The typing procedures used were those outlined by the American Association of Blood Banks and procedural methods of the respective manufacturers.

Determination of Probability of Paternity (PP) and Paternity Index (PI)

The analysis of sperm method as described by Walker was used in calculating the PP and PI, using the appropriate gene frequency tables.

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§ Gamma Biologicals, Inc., Houston, TX.
¶ Ortho Diagnostics, Raritan, NJ.
|| Accugenics, Garden Grove, CA.
Results

The results of HLA typing of the presumed mother, child and parents of the deceased AF in Case #1 are presented in figure 1. From the HLA typing results, the mother and child were found to share the HLA-A2, B7 haplotype. The HLA-A1, B14 haplotype is the obligatory gene complex which had to be donated by the biological father of the child. Following HLA typing of the paternal parents, it was found that the paternal father’s phenotype contained the HLA-A1, B14 markers, thus including the AF as a potential father of the child.

The HLA typing of the paternal parents (all antigens at the A and B loci) results in 16 possible genotypic combinations, four of which contain the obligatory haplotype. These four HLA genotypes which the AF could have expressed are also presented in figure 1. Using these 16 possible genotypes for the deceased AF, the paternity index (PI) was found to be 9:1 with a probability of paternity (PP) of 90 percent. These values for the PI and PP are lower than would be expected if the AF were living and available for testing, since the statistical analysis is derived indirectly using the genetic data of the AF’s parents. The data in table I present the values for the PI and PP of the four genotypic combinations involving the obligatory haplotype, HLA-A1, B14, for the deceased AF if he had been tested directly.

The data presented in figure 2 show the results of HLA typing of the respective parties involved in Case #2. The presumed mother and child were found to share the HLA-A30, by haplotype, indicating that the HLA-A2, B7 is the obligatory haplotype which had to be donated by the biological father of the child. The alleged paternal grandparents do not have this genetic information, however the lymphocytes of the alleged paternal grandmother were found to express HLA-Ax, B7. Although gene mutation and/or suppression is an unlikely event in the HLA system, it is possible that the A2 gene is suppressed. Even though it is highly unlikely that the alleged father is included in paternity, it was not possible to exclude him clearly.

Thus, RBC typing (ABO, Rh, MNSs,
Kk, Fy and Jk) was performed on the respective parties; these results are presented in table II. An exclusion was obtained in the ABO antigen system, since the child typed as an A1B, and neither of the alleged grandparents were able to donate the genetic information for the B antigen. In this case, the deceased AF was excluded from paternity based on the indirect HLA and RBC antigen typing of the paternal parents.

**Discussion**

The results of these two cases corroborate the findings of Spieser et al., in that indirect HLA and RBC typing can be used in cases of disputed paternity when the alleged father is deceased, by testing of the paternal parents. It is important to note, however, that the paternity index (PI) and probability of paternity (PP) values will always be lower when performing this indirect typing, compared to values obtained using the known phenotypes of a living alleged father. In this former case, 16 haplotypic combinations are used in the calculations versus four if the AF was available for typing, thus lowering the PP and PI. The PI and PP in Case #1 were found to be 9:1 and 90 percent respectively; however, the test values would be greatly increased if the AF had been tested directly, as is presented in table I. The PI range would be a low of 21:1 to a high of 208:1, with the corresponding PP values of 95.4 to 99.5 percent based upon the four genotypes containing the HLA-A1, B14 markers.

This indirect typing is also important as a means of excluding an innocent alleged father who is deceased prior to testing, as is presented in Case #2. Following the HLA typing, clear evidence of an exclusion did not exist, since gene suppression could not be ruled out, although the likelihood of it occurring in the HLA system is approximately 1:40,000.* However, following the red blood cell antigen typing, an exclusion

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**TABLE II**

<table>
<thead>
<tr>
<th>Person Tested</th>
<th>ABO</th>
<th>D</th>
<th>C</th>
<th>E</th>
<th>Cm</th>
<th>O</th>
<th>M</th>
<th>N</th>
<th>S</th>
<th>K</th>
<th>K^+</th>
<th>Fy^a</th>
<th>Fy^b</th>
<th>Jk^a</th>
<th>Jk^b</th>
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<td>Alleged grand-father</td>
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<tr>
<td>Alleged grand-mother</td>
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<tr>
<td>Presumed mother</td>
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<tr>
<td>Child</td>
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<td>+</td>
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</table>

*An exclusion; the child expresses the ABO antigen B which had to be donated by the biological father. Neither the alleged grandparents nor mother expresses this antigen.

**TABLE III**

<table>
<thead>
<tr>
<th>Person Tested</th>
<th>ABO</th>
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<th>C</th>
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<th>Cm</th>
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<th>M</th>
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<th>K^+</th>
<th>Fy^a</th>
<th>Fy^b</th>
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<tr>
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</tbody>
</table>

*Presence of RBC antigen; o = Absence of RBC antigen.

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*Personal communication from David Gjertson, B.S., UCLA Typing Laboratory, Los Angeles, California to Robert Gutendorf, M.S., National Paternity Laboratories, Inc.
was obtained in the ABO antigen system, since the child expressed the B antigen which neither of the alleged father’s parents nor the mother expressed. The results of these two studies emphasize the utility of indirect HLA and red blood cell typing in cases of disputed paternity when the alleged father is deceased prior to testing.

As noted in the introduction, our laboratory has been requested on 20 occasions in the past three years to perform this indirect method of paternity testing. Either survivor benefits, such as Social Security, or the potential for estate inheritance from the deceased alleged father are the impetus for this unusual form of paternity testing. Since this indirect form of paternity testing is possible, many more such cases will undoubtedly arise, with many being settled in the court room.

It is imperative to note, however, that an erroneous interpretation can be made if the deceased alleged father’s parents are not the biological parents.

References