LABORATORY EVIDENCE OF UNSUSPECTED PARENTAL CONSANGUINITY AMONG CASES OF DISPUTED PATERNITY

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Summary

A search was conducted to find evidence of possible incestuous unions between the biologic parents of children involved in 2500 paternity cases. Suspicion was raised when either (1) a mother and her child possessed identical HLA phenotypes, or (2) the child appeared to be possibly homozygous for one maternal haplotype (i.e., one of the child's HLA haplotypes was a blank). These mother–child HLA-haplotype dualisms (MHDs) occurred in 8% of all cases. Frequency of exclusion of the accused men in cases demonstrating MHD, was compared with the remaining paternity cases.

No significant difference was found in overall exclusion rates between MHD cases and controls when exclusion produced by HLA and red cell antigen systems were observed. However, there was a greater rate of exclusion in MHD cases when comparing exclusions produced by red cell antigen systems regardless of whether HLA tests excluded paternity ($p < 0.025$). MHD cases involving teenaged mothers differed from control cases in frequency of exclusion of paternity only on the basis of red cell antigen phenotyping ($p < 0.005$).

The HLA system's usefulness in paternity testing is diminished when there is MHD; multiple, independently-inherited systems are relatively more useful in these circumstances.

The search method detects only half of potential incest cases; proof of incest requires more extensive testing for homozygosity among other polymorphisms. Since calculations of likelihood of paternity are inappropriate in cases involving close consanguinity, detection and follow up studies are important.

Data suggest that one-fifth of MHD cases may involve first degree consanguinity and that the incest rate among paternity cases may be as high as 2%.

Introduction

The availability of powerful tests for allelic markers among independent and extensive genetic polymorphisms has greatly enhanced the laboratory's role in resolving problems of disputed parentage [1 - 4]. Two estimates of the power of tests of paternity are the prior probability of exclusion (PPE) and the plausibility of paternity (PP), both of which are calculated from the expected frequencies of occurrence of phenotypes in panmictic populations.

These estimates are based on the assumption of random mating and are erroneous in consanguineous relationships [5, 6]. There are markedly de-
creased PPE and PP for falsely accused men in cases of incestuous father–daughter or brother–sister matings. A test’s effectiveness depends on first accurately identifying the paternal obligatory gene (POG), and then knowing its frequency of occurrence in a known population. Both of these factors are affected by consanguinity.

In the particular case of the usually powerful HLA system, the PPE is reduced by half [5] and the calculated PP is decreased because 50% of the time, 4 POGs are consistent with paternity instead of the usual one: for example, in a father–daughter mating, an HLA haplotype that is infrequent in the population is present in both father and daughter. The example given in Table 1 may serve to elucidate the problem.

Clearly, use of mathematics that assumes random mating in a population with defined frequencies of markers does not apply in cases involving consanguinity. There have been mathematical models proposed for probability of paternity in cases of matings within families, and for probability of incest when the suspicion arises [5, 8]. The problem in the United States, in cases of paternity testing, is that there is often no cause for suspicion. In fact, there are factors that tend to keep cases involving intrafamilial matings outside of the courts:

1. Increased homozygosity of recessive alleles, which occurs in consanguineous matings, is associated with genetic disease and lethal conditions prior to, or shortly after birth [9–11].

2. Access to legal, elective abortion has resulted in early termination of pregnancies of some intrafamilial matings.

3. Mothers involved in such cases may not seek public child support because of the incest taboo.

4. Domestic relations officials often do not attempt to publicly prove paternity when there is strong suspicion of close consanguinity of parents.

Nevertheless, incestuous relations occur in the United States with an estimated incidence of between 0.1 and 500 cases per 100,000 [12]. When a case is tried that involves occult father–daughter or brother–sister relations, the mother might falsely accuse an unrelated man of being father of her child, and she may remain adamant in her accusation. Blood testing often cannot exclude a falsely accused man in such cases, but tends to support the accusation. A priori, expected PPE and PP, calculated on the assumption of panmixis, are both inflated.

Since HLA haplotypes are so numerous, and each is so infrequent, the possession of identical phenotypes in mother and child raises the suspicion of incestuous parentage. Suspicion can be raised in only half of incest cases—those in which the chance occurrence of identical, rare HLA phenotypes in mother and child is exceedingly unlikely in the panmictic population, as compared to intrafamilial matings. Red cell antigens and other marker systems are less polymorphic and their alleles occur more frequently in the population, but these marker systems have special qualities of their own: (a) They may allow demonstration of homozygosity, which is frequent in consanguineous matings [13] and they may permit calculation of probability of
### TABLE 1
Example of father × daughter mating (HLA)

<table>
<thead>
<tr>
<th>Maternal phenotype: A1, A2, B12, B13*</th>
<th>Children</th>
</tr>
</thead>
<tbody>
<tr>
<td>Possible genotypes of offspring</td>
<td>Phenotypes of offspring</td>
</tr>
<tr>
<td>Actual POG by haplotype analysis</td>
<td>Frequency of POG in random population</td>
</tr>
<tr>
<td>Possible POGs consistent with paternity</td>
<td>Frequency of POGs consistent with paternity in a random population</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>A1, B12</th>
<th>A2, A12</th>
<th>A3, B13</th>
<th>A2, A13</th>
<th>B14</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1, A2, B12, B13*</td>
<td>0.005</td>
<td>0.005</td>
<td>0.005</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>A1, B12</td>
<td>0.005</td>
<td>0.005</td>
<td>0.005</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>A1, B12</td>
<td>0.073</td>
<td>0.023</td>
<td>0.005</td>
<td>0.005</td>
<td></td>
</tr>
</tbody>
</table>

*Dualistic phenotypes (see text).
incest [8]. (b) These other marker systems, with few exceptions, are inherited independently. Each system permits some chance of demonstrating an exclusion. Thus, value in excluding falsely accused men is enhanced when any one system fails, no matter how powerful it is. For example, when a child and mother possess similar HLA types (either they are identical phenotypes or the child's phenotype contains a blank haplotype), the mother–child pair is said to have a dualistic haplotype [14]. In these cases, among which there may be consanguinity of mother and biological father, only 1 or 2 second-order exclusions can be made [7]. Often, no exclusion can be made at all. However, the independent assortment of genes of different marker systems permits some chance of finding evidence of a direct or indirect exclusion in each system tested. Given equivalent PPEs there is an advantage in using multiple systems over a single one in excluding the falsely accused [15].

The purpose of this study was to search for evidence that there are court cases in which there is consanguinity between the biological parents of the child. The most likely conditions for close consanguinity involve a dependent female and sexual abuse by an older male, most often the father or brother [8, 12].

Undoubtedly, some mothers have relations with first or second degree relatives because it is an acceptable intrafamilial practice. Incest may occur because family members are intellectually, emotionally, or psycho-pharmacologically impaired. Some persons may be unaware that their sexual partners are family members. Information was unavailable to the laboratory about the social conditions relevant to the cases. It was only possible in all cases to obtain: (1) the mother’s age at the time of delivery of the child, (2) the HLA types of the mother, accused man, and the child, and (3) erythrocyte antigen phenotypes for six blood group systems of the trio: ABO, MNSs, Rh, Kell, Duffy and Kidd.

Method

Hypothesis

When HLA haplotypes of mother and child were dualistic (either they were identical or the child showed one maternal obligatory haplotype and a blank), the possibility of incest was considered. It would be predicted that in incest, the mother would be young, and that there would be an increased proportion of exclusions determined by the less powerful red cell antigen systems (PPE 60%) as compared to HLA (PPE 92%). Also, despite the difficulties in excluding a falsely accused man because of the potentially increased number of possible obligatory genes that may be contributed to the child by men in the population [5], the total proportion of exclusions might be increased in the group of cases exhibiting HLA maternal–child haplotype dualism. (This might occur because (1) the incest taboo causes a much greater proportion of falsely accused men among these cases than in the
remainder, and (2) because the power of the independent tests to exclude the falsely accused is great, despite the 50% reduction caused by the increased frequency of inheriting identical alleles by the children of an incestuous union.)

**Procedures**

2500 consecutive, unselected cases of disputed paternity were studied retrospectively. The Baltimore Rh Typing Laboratory routinely performs HLA and red cell antigen testing on all cases. Together, the HLA and red cell antigen systems ordinarily yield a prior probability of exclusion of 97%. In cases where a single second-order exclusion is found, additional testing of red cell enzyme and plasma protein polymorphisms is undertaken. These additional tests are not part of the routine battery performed by the laboratory and are considered adjunctive; they are used to confirm an indicated exclusion. Methods are further described in a previous publication [15].

Cases were examined for dualism of the HLA haplotype in mother and child. Dualism is defined as either identical phenotypes (two haplotypes) in mother and child (e.g., mother: A1, A2, B13, B15; child: A1, A2, B13, B15) or one identical haplotype in mother and child, plus a 'blank haplotype' in the child, which suggests possible homozygosity (e.g., mother: A1, A2, B13, B15; child: A1, A-, B13, B-). The number of cases with maternal haplotype dualism was compared with the number of cases of paternal haplotype dualism to see if more maternal than paternal dualism cases were present. If dualism was simply a chance event, it would involve each of the biological parents with equal frequency.

Since it is assumed that the mother is the biological mother of the child, but that the putative father may not be the biological father of the child, adjustment was made for: (1) the actual exclusion rate obtained for all cases investigated by the laboratory (26%) and (2) the expected rate of failure to exclude (3%) based on the known prior probability of exclusion (PPE 97%) [15]. Furthermore, comparison of cases of maternal-child haplotype dualism versus those cases without dualism should yield equivalent rates of red cell exclusions if haplotype dualism is a chance event. If maternal haplotype dualism (MHD) is related to an increased frequency of inheritance of identical HLA alleles by the child, caused by close consanguineous union of the mother, then a significantly greater proportion of red cell exclusions of paternity should result, as compared to the rest of the cases. Sub-groups selected for comparison with the remainder of the cases were: (1) cases of MHD, (2) cases of MHD in which the mother was 19 years old or less at the time of delivery of the child, and (3) cases of MHD with mothers of less than 19 in which the combined frequency of the 4 HLA paternal obligatory genes in the population was less than 0.05 ('low frequency'). These sub-groups were compared to the remaining cases with respect to number of exclusions by (a) all methods, (b) red cell antigen phenotypes (regardless of whether there was an HLA exclusion or not), and (c) red cell antigens without HLA. Differences in numbers of exclusions in each group were evaluated statistically by chi square analysis.
Results

Results are summarized in Fig. 1.

In the 2500 cases reviewed, the number of exclusions, based on both red cell and HLA studies, was 637 (25.5%). The number of red cell exclusions was 342 (13.7%), but, as expected, in most cases where red cell exclusions were found, HLA also excluded the alleged father from paternity. Only 23 cases (1.0%) were found in which red cell antigen tests excluded paternity but HLA did not.

There were 125 cases (5.0%) of maternal haplotype dualism and 83 cases of paternal haplotype dualism.

There were 637 men excluded from paternity. The number 637 accounts for exclusions expected from the prior probability estimate of 0.97. Theoretically, there are 656 men who are not the fathers of children among the cases and would be excluded if the tests were perfect (PPE = 100%).

Thus, of 2500 cases, 2044 is the number of men who should be included as fathers (2500 - 656), and 83 of those showed maternal haplotype dualism (4.06%).

This proportion 83/2044 is lower than the counted number of cases of maternal haplotype dualism (125/2500), and is consistent with the possibility that there may be a few trios among paternity cases that are inbred (23/2500 = 1%), however, the data are not statistically significant.
Among the cases of maternal haplotype dualism, HLA and red cell blood groups excluded 35 (28.0%) alleged fathers from paternity. Red cell antigen tests excluded 24 men in total (19.2%). This is significantly greater than in the cases without MHD ($p < 0.025$). Red cell tests alone excluded 6 men (4.8%). This proportion of cases is significantly higher than is observed in the rest of the cases in the study population ($p < 0.05$).

There were 48 cases of maternal haplotype dualism in which the maternal age at the time of delivery was 19 years or less. In this group, there were 17 cases of exclusion of paternity (35.4%) based on HLA and red cell antigens. Red cell tests excluded 11 men (22.9%). Red cell tests alone excluded 2 (4.2%). The last proportion is statistically significantly greater than the remainder of cases ($p < 0.005$). (In order to rule out the possibility that teenaged mothers falsely accused men more often than other mothers, 50 non-MHD, teenaged cases were selected. Exclusions rates were 24% for all exclusions, 14% for red cell exclusions with or without HLA, and 2% for red cell exclusions only. These proportions are similar to other cases and lower than cases with MHD and teenaged mothers.)

There were 14 cases in which there was maternal haplotype dualism, the mother was 19 years old or younger at the time of delivery of the child, and the frequency of the combined 4 paternal obligatory genes for HLA in the general population was 0.05 or less. In this group, HLA and red cell serologic tests excluded 7 men from paternity (50.0%). Red cell tests excluded 5 of the 7, but there were no cases in which red cell tests alone excluded the putative father from paternity (see Fig. 1).

Discussion

It is twice as difficult to exclude paternity when the child and mother possess identical haplotypes [5]. The probability of excluding from paternity a man chosen at random from the population, can be calculated from the frequencies of the genes of the independent systems evaluated in testing [6]. If a gene occurs infrequently, as does each HLA gene, and the child possesses the gene, the chances of excluding a falsely accused man drawn randomly from a panmictic population, are great. However, the chances of exclusion from paternity are dependent on accurately defining the paternal obligatory gene from the phenotypes of mother and child, and then calculating the proportion of the population which does not possess the obligatory genes. When the mother and child possess identical phenotypes, or the child has an HLA phenotype consisting of the maternal obligatory gene and a blank haplotype, a paternal obligatory gene cannot be clearly identified so there is an increased proportion of the male population which could contribute any one of up to four genes.

Furthermore, when an accused man is not excluded on the basis of HLA testing, it is ordinarily anticipated that the infrequency of encountering each HLA gene in the population will serve to increase the chances of in-
cluding the putative father when he and the child both appear to have a given haplotype. When an incestuous union of the mother produces a child, and a random man is falsely accused, there is a 50% chance that the child either will have a phenotype that is identical to the mother (25%) or will share one haplotype with the mother but demonstrate no second haplotype—a blank (25%). In these 50% of incest cases there is no sharing of a demonstrable, infrequent gene between child and falsely accused man. Thus, the calculated paternity index or plausibility of paternity values are decreased in magnitude.

After adjustments are made for test failure rate and for exclusions, and despite the potentially lethal effects of homozygosity of HLA genes [9 - 11], or genes of other systems, there remains a slightly greater number of cases of maternal haplotype dualism as compared with paternal haplotype dualism. Although this difference of about 23 cases per 2500 studied is not statistically significant, the methods used are able to detect only half the cases. Another 50% of incest cases might not result in the presence of MHD (see children 3 and 4 of Table 1). The chances of excluding a falsely accused man, using the HLA system, would be equal to that in the general population in these non-dualistic incest cases. Thus, 46 cases per 2500 is an estimate of close consanguinity among court cases, if each case was a mating of first degree relatives (e.g. father–daughter). Union of more distant relatives (e.g. uncle–niece, first cousins) is less socially stigmatic and decreases the social pressure to falsely accuse someone. Such a union would result in MHD with a frequency closer to that of random matings. For example, the chance of homozygosity for HLA of the child of incest is 25%; it is 12.5% for an uncle–niece mating and only 3% to 6% for cousin matings.

Since there is 50% devaluation of the power of a test in excluding paternity on the basis of any single genetic system when MHD is seen, it is important to utilize a number of genetically independent marker systems.

When there is a sharing of two HLA haplotypes between mother and child or when the mother shows two distinct haplotypes and the child shows one in common with the mother plus a blank haplotype, consideration might be given to the possibility that the child may be an offspring of a consanguineous relationship. From further analysis of the data, suspicion should be increased when such maternal haplotype dualism is associated with a mother who is in her teens, and possibly, when the paternal obligatory gene is of very low frequency in the population. These findings are supportive of studies by others who have used different methods [10, 12]. Actual proof of incest depends on demonstration, in a child, of homozygosity in a number of independent genetic systems and calculation of the probability of their occurring simultaneously by chance.

Results suggest that as many as 2% of paternity cases may involve a falsely accused man and mating of first-degree relatives who are the biological parents of the child. Half of these cases may be suspected because the child and mother share HLA haplotypes. Such MHD dualism is seen in 5% of all cases, among which four-fifths are certainly the result of chance matings.
Close consanguinity is more likely when such dualism is accompanied by teenage motherhood. Cases involving close consanguinity of parents decreases the likelihood of exclusion (PPE) of a falsely accused man when the HLA system is used alone. There is also a decrease in the calculated values of plausibility of paternity and paternity index. Additional testing, especially for independent markers in other polymorphisms (such as red cell antigens) provides the means of exonerating a falsely accused man. Some systems that demonstrate homozygous states also offer the possibility of calculating the probability of incest. When a single paternal obligatory gene cannot be ascertained, the usual methods of calculation of probabilities must be questioned.

References


