CASE REPORT

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Linking a Bloodstain to a Missing Person by Genetic Inheritance

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ABSTRACT: The use of the principles of genetic inheritance to link a bloodstain to a missing victim is discussed. Genetic marker typing can determine whether the parents of the missing victim are possible parents of the bloodstain source. Given a parental inclusion, it is possible to calculate the probability that a randomly selected couple would possess the necessary genetic combinations to be the parents of the person who bled. The applications of this concept using several genetic marker systems are examined. General formulas have been developed for the probability calculation using phenotype distribution frequencies and gene frequencies. This approach was applied to a homicide case in which it was shown that the victim's parents were among only 0.8% of couples from the general population who would be able to bequeath the genetic marker types found in the bloodstain. This evidence was helpful in producing a conviction of first degree murder.

KEYWORDS: pathology and biology, genetic typing, blood, paternity testing

In most forensic serology cases blood standards from the suspected sources are available for comparison to bloodstains found at the scene or on a suspect. There are, however, exceptions. A homicide victim's body may have been devoured to the bare bones by wild animals or may be found in an advanced stage of decomposition. In other cases, the body might have been disposed of so that it is never found, for example by being burnt to ashes or dumped in the ocean. Such cases might nonetheless involve a scene, a vehicle, or a suspect with well-preserved bloodstains, possibly from the victim. Attempting to associate these bloodstains with the victim has previously been limited to the scanty typing information that can be developed from what remains exist [*1,2*] or from stains on articles known to be the victim's. This paper describes a completely independent approach to this problem: the use of genetic inheritance testing to link the victim's parents to bloodstains believed to originate from a missing victim.

The inducement to develop this approach came from a homicide case. A young woman was reported missing in August 1979. Kidnapping and extortion were apparently involved. A suspect was located by police investigators. He admitted to participating in the kidnap plot but denied knowledge of what might have happened to the victim. He claimed that two other men

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took her out to sea in his boat and later returned without her. The authorities were unconvinced. The suspect was arrested and charged with the kidnapping.

Search warrants were served on the suspect's residence and boat. In the attic of his apartment police found a T-shirt and pair of trousers with bloodstains on them. On the boat a fine spray of dried blood was found on the vinyl upholstery of the seat cushions. All these articles were seized and brought to the laboratory for analysis.

The blood typing results are listed in Table 1. It is clear that the bloodstains on the clothing and boat seat did not originate from the suspect. Moreover, the combination of types found in the blood on the boat is rather uncommon. An individual having this combination of phenotypes for these six genetic markers occurs with a frequency of about 1 in 6000 in the general population.

Seven pairs of soiled women's ~~un~~derpants were retrieved from the victim's residence. Crotch areas were examined. Two pairs that were semen-free had faint blood streaks sufficient for ABO typing only. The blood on each pair of underpants was determined to be Type B. While this showed that the victim had a blood type consistent in one group with that of the blood on the boat seat, so would approximately 10% of the population.

Days went by and the victim's body was not found. Faced with the probability that her body would never appear (the anchor from the boat was also missing), the investigators sought another way of linking the blood on the boat to the missing victim.

The approach taken was to apply the principle of genetic inheritance testing. The parents of the missing victim were accessible and it could be determined whether their blood types were included as possible parental types for the individual who bled on the boat. The significance of an observed parental inclusion could then be assessed by determining the probability that a randomly selected couple might meet the requirements for parental inclusion, that is, by determining the frequency of included parental couples in the population.

To illustrate this approach, general situations for several types of genetic marker systems are presented below. Formulas are derived for calculating the probability P that a randomly selected couple would meet the criteria to produce a child of a particular type.

Assessment of Parental Inclusion

Two-Allele System, Codominant Expression

Consider first a genetic marker system with two codominant alleles a and b. The possible phenotypes are A, AB, and B, with frequencies denoted by $\overline{A}$, $\overline{AB}$, and $\overline{B}$, respectively. Table 2 illustrates the possible mating combinations for progeny of each phenotype.

TABLE 1—*Blood typing results on bloodstains.*[a]

Sample Source	ADA	AK	Hp	PGM	EAP	ABO
Suspect's standard	1	1	2-1	2-1	CB	0
Bloodstain on boat seat	1	2-1	2-1	2-1	A	B
Bloodstain on suspect's trousers	inc[b]	2-1	ND[c]	inc	inc	B

[a]ABO type was determined by the absorption-elution technique [3]. Phosphoglucomutase (PGM) type was found by using the method of Wraxall [4]. Red cell acid phosphatase (EAP), adenylate kinase (AK), and adenosine deaminase (ADA) were determined by using the buffer system of Polesky [5] and the method of Hopkinson [6]. Haptoglobin (Hp) was determined by vertical gradient polyacrylamide gel electrophoresis using Pharmacia gel PAA 4/30 and electrophoretic apparatus GE-4. Esterase D (EsD) and glyoxylase I (GlOI) typing were attempted on the stains with inconclusive results.

[b]Results inconclusive.

[c]Test not performed.

TABLE 2—*Possible mating combinations for progeny of each phenotype.*

Progeny	Parent 1	Parent 2
A	A or AB	A or AB
B	AB or B	AB or B
AB	A or AB	AB or B
AB	AB or B	A or AB

For a child to be homozygous A, each parent must have at least one *a* allele. The probability that a randomly selected individual possesses an *a* allele is $(\bar{A} + \overline{AB})$, and the joint probability of selecting a mating couple that meets this requirement is given by:

$$P_1 = (\bar{A} + \overline{AB})^2 \tag{1}$$

Similarly, for a homozygous B child, the probability is:

$$P_2 = (\bar{B} + \overline{AB})^2 \tag{2}$$

For a child to be heterozygous AB, one parent must have at least one *a* allele and the other parent at least one *b* allele. This could occur two ways—male *a*/female *b* or vice versa—and thus the frequency term needs to be multiplied by a factor of two. This doubling includes a redundancy for both parents being AB and this needs to be subtracted out. The final equation is:

$$P_3 = 2(\bar{A} + \overline{AB})(\bar{B} + \overline{AB}) - (\overline{AB})^2 \tag{3a}$$

An alternate way to calculate this probability is by stipulating that both parents cannot be homozygous A and both parents cannot be homozygous B. Thus:

$$P_3 = 1 - (\bar{A}^2 + \bar{B}^2) \tag{3b}$$

It is easily shown that Eq 3b is an algebraic equivalence of Eq 3a.

The above equations can be algebraically reformulated in terms of gene frequencies. If it is assumed that p is the gene frequency of allele *a*, q is the gene frequency of allele *b*, and $p + q = 1$, the resulting equation for a homozygous A child is:

$$P_1 = (p^2 + 2pq)^2 = p^2(2 - p)^2 \tag{4}$$

For a homozygous B child the equation is:

$$P_2 = q^2(2 - q)^2 \tag{5}$$

And for a heterozygous AB child it is:

$$P_3 = 2pq[(2 - p)(2 - q) - 2pq] \tag{6}$$

Three-Allele System, Codominant Expression

Now consider a system with three alleles *a*, *b*, and *c*. The common phenotypes are A, AB, AC, B, BC, and C, and the frequency notation is as above. To produce a homozygous A child, both parents must possess at least one *a* allele; the probability of this occurrence is:

$$P_4 = (\bar{A} + \overline{AB} + \overline{AC})^2 \tag{7}$$

This may also be expressed in terms of gene frequencies, with r denoting the frequency of allele c:

$$P_4 = (p^2 + 2pq + 2pr)^2 = p^2(2 - p)^2 \tag{8}$$

Note that Eq 8 is identical to Eq 4 for the two-allele system. For a heterozygous child, such as an AB, the same logic applies as for Eq 3a:

$$P_5 = 2(\bar{A} + \overline{AB} + \overline{AC})(\bar{B} + \overline{AB} + \overline{BC}) - (\overline{AB})^2 \tag{9}$$

or

$$P_5 = 2pq[(2 - p)(2 - q) - 2pq] \tag{10}$$

It should be pointed out that the logic of Eq 3b does not apply with three-allele systems.

Multiple-Allele Systems, Codominant Expressions

From the foregoing discussion it is evident that the probability equations can be expressed in general terms applicable to codominant systems containing any number of alleles. For a homozygous child of phenotype A where the frequency of the a allele is p:

$$P_A = (\bar{A} + \overline{AB} + \overline{AC} + \overline{AD} + \ldots)^2 \tag{11}$$

or

$$P_A = p^2(2 - p)^2 \tag{12}$$

For a heterozygous child of phenotype AB, where the frequencies of a and b are p and q, respectively:

$$P_{AB} = 2(\bar{A} + \overline{AB} + \overline{AC} + \overline{AD} + \ldots)(\bar{B} + \overline{AB} + \overline{BC} + \overline{BD} + \ldots) - (\overline{AB})^2 \tag{13}$$

or

$$P_{AB} = 2pq[(2 - p)(2 - q) - 2pq] \tag{14}$$

The ABO System

The ABO system is considered separately since it involves dominant and recessive expression. To produce a Type A child, at least one parent must be A or AB. The probability is expressed as:

$$P_6 = (\bar{A} + \overline{AB})^2 + 2(\bar{A} + \overline{AB})(\bar{O} + \bar{B}) \tag{15}$$

The first term of the equation covers the situation where both parents are either Type A or Type AB, and the second covers the situation where only one parent is an A or AB. (Although a homozygous B/B individual obviously cannot be the parent of an A child, genotyping is not commonly done in ABO testing and B/B individuals are not distinguished from B/O individuals in the B frequency term $\bar{B}$.) Similarly, for a child of Type B:

$$P_7 = (\bar{B} + \overline{AB})^2 + 2(\bar{B} + \overline{AB})(\bar{O} + \bar{A}) \tag{16}$$

For an O child, neither parent can be Type AB:

$$P_8 = 1 - [(\overline{AB})^2 + 2(\overline{AB})(\bar{A} + \bar{B} + \bar{O})] \quad (17)$$

For an AB child, one parent must be A or AB and the other B or AB:

$$P_9 = 2(\bar{A} + \overline{AB})(\bar{B} + \overline{AB}) - (\overline{AB})^2 \quad (18)$$

which is identical to Eq 3a.

Application

To evaluate the bloodstains on the boat, the frequency of possible parental couples in the population was calculated by using the appropriate equations developed above (see Table 3). The cumulative probability that a randomly selected couple would possess the necessary traits to produce an offspring with the blood types found on the boat was calculated as 0.0077; in other words, about eight in a thousand randomly selected couples would possess the necessary traits by those six genetic markers to qualify as parents of the person whose blood was found on the boat.

Subsequently, blood standards were obtained from the victim's parents. Their blood types are listed in Table 4, along with those from the stain on the boat seat. Obviously, the victim's mother and father possess the necessary genetic traits and belong to that 0.77% of random couples who qualify to be the possible parents of the person who bled. This finding, in conjunction with other evidence presented at the trial, contributed to the conviction of the suspect.

TABLE 3—*Phenotype frequencies for Orange County.*[a]

Marker System	Orange County Phenotype Frequencies	Bloodstain Type	Applied Equation	Progeny of Parental Couples
ADA	$\bar{1} = 0.87$	1	1	1.0
	$\overline{2-1} = 0.13$			
	$\bar{2} < 0.01$			
AK	$\bar{1} = 0.91$	2−1	3	0.172
	$\overline{2-1} = 0.09$			
	$\bar{2} < 0.01$			
Hp	$\bar{1} = 0.18$	2−1	3	0.859
	$\overline{2-1} = 0.49$			
	$\bar{2} = 0.33$			
PGM	$\bar{1} = 0.60$	2−1	3	0.637
	$\overline{2-1} = 0.35$			
	$\bar{2} = 0.05$			
EAP	$\bar{A} = 0.11$	A	7	0.314
	$\bar{B} = 0.41$			
	$\bar{C} < 0.01$			
	$\overline{BA} = 0.42$			
	$\overline{CB} = 0.03$			
	$\overline{CA} = 0.03$			
ABO	$\bar{A} = 0.39$	B	16	0.260
	$\bar{B} = 0.10$			
	$\bar{O} = 0.47$			
	$\overline{AB} = 0.04$			

[a]Based on case work data developed by the Orange County Sheriff-Coroner Laboratory.

TABLE 4—*Blood typing results for victim's parents.*[a]

Sample Source	ADA	AK	Hp	PGM	EAP	ABO
Blood from boat	1	2-1	2-1	2-1	A	B
Victim's mother	1	2-1	2	1	BA	O
Victim's father	1	1	2-1	2-1	BA	B

[a]Typings were performed as described in Table 1.

Discussion

The concept of linking a bloodstain to a missing body through genetic inheritance testing is presented. The applicability of the formulas developed is illustrated by their use in a homicide case. The usefulness of this technique obviously depends on the extent of typing information available from the stain and on the frequency of the blood types found in the stain. Obviously, the more markers that can be employed and the rarer they are, the higher the exclusion rate will be and the greater the weight of the association. It is also vital to ascertain true parentage, otherwise false inclusions or exclusions could result.

A similar approach could be applied in cases where the missing person is married with children; information could be derived by examining the blood types of the offspring and the spouse. The logic to follow in such cases would be analogous to that employed in paternity testing [*7–10*]. As above, the extent of typing information, the frequency of the types, and the authenticity of information on parentage determine the usefulness of the approach.

In summary, in the absence of exemplar blood samples to compare to a bloodstain of unknown origin, it is still possible to derive useful information if close relatives of the suspected stain source are available for typing.

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