DETERMINATION OF GENOTYPES BY FAMILY STUDIES

Report of Five Illustrative Cases

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HUMAN blood groups are unique among inherited physical characteristics in that each blood group factor is transmitted by the mode of single-gene inheritance. This means that the inheritance of blood group factors is determined by a single set of allelomorphic genes. In contrast to this, physical characteristics such as skin color and eye color have a much more complex mode of inheritance and may require many genes in order to produce a specific inherited character. Because the inheritance of blood group factors is so comparatively simple, geneticists have found them a convenient group of characteristics to employ in various types of genetic studies.

A second characteristic of blood groups, which makes them particularly useful in the medical field, is that in the most usual form of inheritance the genotype of an individual can be determined as well as his phenotype. Thus, the type MN person has inherited a gene for M from one parent and a gene for N from his other parent. The genes for M and N are codominant and the heterozygote is readily detected by laboratory tests. A type M person is genetically homozygous for M and must have inherited a gene for M from each parent.

Unfortunately, there are exceptions to this simple inheritance of blood group factors by codominant genes, and when they occur it is necessary to utilize means other than the conventional laboratory procedures to determine genotypes in two of the most important blood group systems, i.e., the ABO and Rh-Hr systems. In the ABO system, the genes for A and B are codominant and when present are revealed in the individual phenotype. The group AB person carries a gene for A and a gene for B. However, group A or group B persons may be homozygous or heterozygous for A or B and there is no way in the laboratory to tell the difference. In the Rh system, the antisera which are used to determine the presence of each of the Rh-Hr antigens are obtained from patients who have had diseases related to incompatibilities in the Rh-Hr system. Unfortunately, while antisera for C, c, D, E, and e are available, antisera for d are not. The presence of d is determined by the absence of D, so that the rh-negative (dd) individual can be identified only in the absence of D. The Dd heterozygote cannot be distinguished from the DD homozygote.

Information about a husband's genotype may be of considerable comfort

to both the husband and the wife in a family who has a problem of an erythroblastotic pregnancy. Indeed, such information may be of real help in many aspects of family planning. In the past, the problem of determining the genotype of the father of a child affected with Rh-induced erythroblastosis has been met by determination of the phenotype of the man, and reference to available published tables of genotype frequency. This is a useful procedure, and in many instances is useful in helping a family to make a decision whether to avoid or to try to have more pregnancies. Adoption proceedings, artificial insemination, and sterilization procedures may depend upon such reports. While such efforts are useful, it is unfortunate that more recourse is not made to family studies to help to clarify the problems of genotypes. Such family studies do not stop with the blood bank's report on the Rh type of the affected infant's blood, but should consider the phenotypes of the parents, of all the children in the family, and of the grandparents, parental siblings, and other members of the family. The value of this approach is shown in the five cases presented in this report.

The problems encountered in attempting to determine the genotypes of fathers of children afflicted with erythroblastosis differ with each of the blood group systems ordinarily involved in this disease. In hemolytic disease of the newborn, of ABO incompatibility, the problem revolves around the inability of laboratory tests to distinguish between persons who are heterozygous either for A or for B, and persons who are homozygous for either of these factors. The differentiation is quite important and has vast implications in the counseling and management in regard to the disease of the patients. The problem becomes even more acute when one realizes that the most common situation in which ABO erythroblastosis occurs is one in which the father is group A and the mother is group O. In such situations, it is important to be able to inform the couple what the chances are that they will have a normal baby or a defective baby, and their chances of being able to have a normal child in future pregnancies. In the Rh-Hr subtypes, the problem lies in the inability of laboratory tests to distinguish the individual who is heterozygous from the one who is homozygous for D. The published tables for frequencies of genotypes of this blood type system give us much information that is helpful, but, as shown in one case, sometimes at least, this information may lead to erroneous conclusions. There is no problem in regard to the other Rh-Hr factors, as it is possible to determine both allels of C and E.

This report is concerned with the attempt to use family studies of blood groups as an aid in solving the dilemma. It was stimulated by the problems presented by case 1, and has had useful application in a number of subsequent cases.

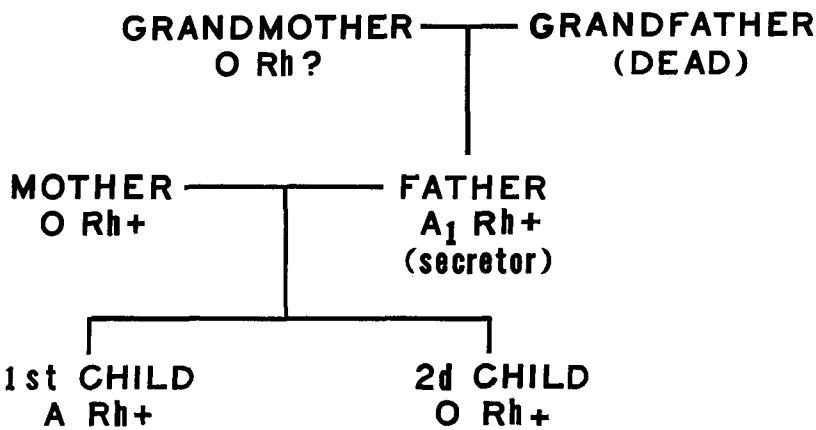

Fig. 1. Case 1. Family blood group pedigree.

REPORT OF ILLUSTRATIVE CASES

Case 1. A 43-year-old woman was first examined in July 1964 at the Cleveland Clinic because of her pregnancy. Her only previous successful pregnancy had resulted in an erythroblastotic son who was then 15 years old and so greatly retarded as to require care in an institution. This child's blood type is group A, Rh positive; the mother's group is O, Rh positive; the father's is group A_1, Rh positive, and he is a secretor. The mother showed a higher titer of anti-A in her blood throughout the pregnancy, and it was anticipated that she would have the same problems with this next child as she had had with the previous one. The family consulted me for whatever reassurance I could offer them about the possibility of a successful outcome of the present pregnancy. The family history (*Fig. 1*) taken at that time revealed that the patient's mother-in-law was alive and that her blood group is group O; the patient's father-in-law is dead, and his blood group is not known. This means that the patient's husband must have a genotype of AO and that, in spite of the high titer for anti-A displayed by his wife, there was statistically an equal chance that the infant would or would not be defective. Subsequently, the patient delivered a full-term, male infant whose blood group proved to be O, and who, at 10 months, appears to be perfectly healthy. The high titer for anti-A shown in the mother's blood during the pregnancy cannot be readily explained.

Case 2. This case concerns the Rh-Hr subtypes, and illustrates the value of accumulating more data to make genotype estimations more precise than can be determined from the published frequency tables. The problem of genotypes in the Rh-Hr system is best known and merits little discussion, except the reminder that most blood banks that report Rh-Hr typings on newborns are reporting only on the D (Rho) factor.

A 30-year-old physician in June 1964 requested a genotype study of himself because of an erythroblastotic birth in the family. His cells were found to become agglutinated with anti-C, anti-D, anti-c, and anti-e serum but not with anti-E. He was told that his probable genotype was CDe/cde and that he had an excellent chance of being the father of an rh negative child. This was in accordance with the statistical frequencies that are published in regard to Rh-Hr subtypes. Subsequently his wife accompanied by their only living child, a son, was seen. A specimen of blood was taken from the son for determination of his genotype. The child's blood proved to react with anti-D, anti-c, and anti-e, but not with anti-C or anti-E. His probable genotype was, by serologic tests, cDe/cde and this was confirmed by the fact that his mother was rh negative (cde/cde) and hence could transmit only the cde gene to her son. This meant that he received a cDe gene from his father, whose genotype then must most probably be CDe/cDe or cDe/Cde, as CDe/cde is excluded by the observation of the

Table 1.—*Blood group data on family in case 2*

Member of family	Reactions with antisera for					Possible Rh-Hr genotypes by serologic tests	Calculated genotype frequency,[1] percent
	C	D	E	c	e		
Mother	−	−	−	+	+	cde/cde	15.10
Father	+	+	−	+	+	CDe/cde	32.68
						CDe/cDe	2.16
						cDe/Cde	0.05
Child	−	+	−	+	+	cDe/cde	1.99
						cDe/cDe	0.07

child's blood (*Table 1*). As the frequency of CDe/cDe is 2.16, while the frequency of the alternate possibility of cDe/Cde is only 0.05, or a ratio of more than 1 in 40, there is little possibility of this couple's having any rh negative children. This revised the optimistic conclusion of the initial findings and further pregnancies were not recommended.

A full-term infant, who had been the couple's first child, was born alive and died a few hours after birth, was also reported as Rh positive. If further information in regard to the Rh-Hr system in the dead child were available, it is possible that the genotype of the father could be determined with even more certainty.

Case 3. This case illustrates the unreliability of accepting outside sources of medical information and also the problem of not always being able to obtain the full cooperation of the family. The couple had had several unsuccessful pregnancies, and now had two living children. They consulted me in August 1965 because of Rh incompatibility, and the difficulties were ascribed to ABO erythroblastosis. The father's blood group was A, Rh positive, and the mother's blood group was O and also Rh positive. Since the woman was pregnant, a family study seemed indicated to determine whether or not there was hope of their having a normal child. The appointment was made for them, but at the last minute the husband decided that his children might become "frightened," or "be hurt" by having blood drawn, and he insisted that the blood group data be utilized from the two hospitals where the children had been born. When the reports were received it was found that one child was group A, Rh positive, but the second child was reported as group AB, Rh positive. This was a clear case of maternity exclusion, so that either the data sent to us were incorrect or some mistake had been made in the hospital nursery.

Case 4. This case concerns a family in whom the ABO genotypes of nine members of the family were accurately determined by the family study carried out from June to August 1964. The family consists of five group A, one group B, three group AB, and one group O members. The genotypes of all of the group A and group B members of this family were established with certainty except for one man; whereas without family studies, these genotypes would only be subject to guesses. *Figure 2* shows the distribution of the genotypes of the nine persons as determined by family studies.

Case 5. This family was seen in February 1966 when they came to the Cleveland Clinic Hospital to take home an 8-month-old child who had been brought here for evaluation. The husband and wife had two children. The first of these was a normal, Rh positive boy. The second, also a boy, was Rh positive and had been severely erythroblastotic at birth. In spite of exchange blood transfusions the child was left severely retarded. The parents wanted advice regarding the desirability of further pregnancies. The mother was rh negative, and stated that both her mother and her grandmother were rh negative. The father was Rh positive and stated that his mother was rh negative. This information solved their problem, as the father most certainly had to be heterozygous and further pregnancies resulting in normal children seemed possible. Blood specimens were obtained from several

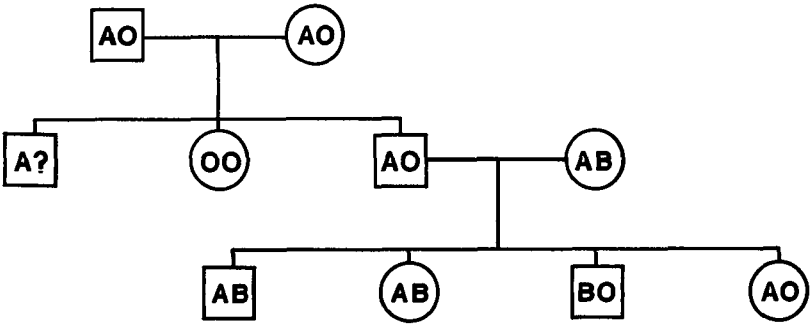

Fig. 2. Case 4. Genotypes of nine members of the family.

Table 2.—*Blood group data on family in case 5*

Member of family	Blood group	Reaction to anti-Rh sera					Possible Rh-Hr genotypes	Published frequency,[1] percent
		C	D	E	c	e		
Mother	O	−	−	−	+	+	cde/cde	15.10
Father	O	+	+	−	+	+		
First child	O	+	+	−	+	+	CDe/cde	32.68
Second child	O	+	+	−	+	+	CDe/cDe	2.16
Paternal grandfather	O	+	+	−	+	+	Cde/cDe	0.05
Paternal grandmother	A	−	−	−	+	+	cde/cde	15.10

members of the family, and the assumption made from the information supplied by the father was happily confirmed (*Table 2*).

Without the information about the paternal grandmother it is possible only to guess at the genotype of the father. As a result of this family study each person's genotype is known with certainty, with the exception of the paternal grandfather whose genotype could be resolved by resorting to data from his own parents, siblings, or other children. Using only the serologic data obtained from studies of the father's blood, there would have been an 88 percent chance of being correct in reporting him as heterozygous for D.[1] Using the family data, it is obvious that this is now a certainty.

DISCUSSION

A review of my own unpublished data on 650 paternity exclusion studies indicates that there are few instances in which a family study would have been of help. Several attempts to enlist the aid of individuals involved in such situations met with lack of cooperation, and sometimes hostility on the part of the persons most concerned.

Recently Allen[2] discussed the use of the blood groups of relatives of the principals in paternity tests. He presented two cases in which blood typing tests of relatives provided critical evidence regarding the genotypes of the defendants in bastardy proceedings. In one instance, the tests confirmed the supposition of nonpaternity, and in another case, the use of family studies refuted the presumption of nonpaternity. However, tests of relatives of individuals involved in legal proceedings of paternity cases are subject to criticism in that the reliability of the word of these relatives is probably as much open to question as that of the individuals who are the principals of such suits. In addition to this, the number of instances in which useful information in paternity exclusions is obtained are relatively few, as it is usually most helpful to the accused to demonstrate homozygosity; whereas family studies, when successful, most often demonstrate heterozygosity. Both of Allen's[2] cases are curious. The first depended upon the rare Penney allel of the Kell system, and in the other study, the original presumption of an exclusion was made on what appears to be questionable grounds.

These objections usually do not apply to determinations of genotypes of patients with blood group incompatibilities that complicate pregnancies. The families usually are willing to be cooperative. Also, in obstetric studies, it is usually more helpful to the patient to be able to demonstrate heterozygosity rather than homozygosity, and, as indicated above, this can be most easily done using the technic of family studies.

Approximately 50 percent of group A or group B persons are heterozygous for O, and with a suitable marital partner potentially could be the parents of group O offspring. As most ABO erythroblastotic problems occur with the group A father and group O mother, the possibility that a child of such a mating will be group O is about one in four, which are undesirable odds to a family especially desirous of having children. However, these odds can be increased, if it can be shown that one of the parents of the group A father is either group O or group B; under such circumstances the father must be heterozygous for A. In the relatively unusual circumstances of both the paternal grandparents being group AB and the father himself in group A, homozygosity for A must be accepted as definite. If both paternal grandparents are group A, little can be said about the genotypes of their offspring, unless heterogeneity can be shown by studies of any other siblings. Hence, if the parents are heterozygous for A, there is an excellent chance that the person too is heterozygous for A.

Information about the progeny of a family involved in ABO erythroblastosis is usually on the hospital chart, but the determinations may be based on cord blood and should be rechecked when the infants are at least six months old. If a family has other group O children, they can have another group O child, and the problem, as far as making recommendations to the family is concerned, is solved.

In the second family considered, the father's blood reacted to antisera for the factors C, D, c, and e, but not for E (*Table 1*). Referring to published tables, it can be seen that 32.68 percent of Caucasians have the genotype CDe/cde; 2.16 percent have the genotype CDe/cDe; and only 0.05 percent have cDe/Cde. The obvious educated guess as to the father's genotype then becomes CDe/cde, the odds in favor of this being correct being about 15 to 1. The data on the child's genotype show that, using the serologic data above, the child's genotype could be cDe/cde (representing 1.99 percent of Caucasians) or cDe/cDe (representing 0.07 percent). However, as it is known that the child's mother is rh negative it must be concluded with absolute certainty that the child is actually cDe/cde, having obtained the cde gene from his mother and the cDe gene from his father. If the father gave his son a gene for cDe he must not be CDe/cde, even though the odds for this are 15 to 1, but must be either cDe/Cde or CDe/cDe. If the first situation exists, then this man could be father of a child whose genotype is negative for D and hence possibly normal; however, the odds are greater than 1 to 40 against this occurrence.

The newer blood groups, such as the Lutheran, the Kell, and the Duffy blood group systems, offer relatively little difficulty, because in both the Kell and the Duffy systems there are antisera that react with all known subgroups. Thus, an individual who is Fy^a positive can be detected by reacting positively with Fy^a antiserum, and unless he reacts with Fy^b, he has to be considered negative for this antigen. However, because of the newness of our knowledge of these blood groups and the relatively low frequency of such reactions, it is probable that family studies will be of little help in these problems for some time to come.

SUMMARY

The application of family studies of blood in the determination of genotypes of blood group factors is an easy technic employing only facilities that are available in the average adequate clinical laboratory or blood bank. The added information may be of great benefit to the peace of mind of the anxious patient and can play an important role in the decision about family planning.

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