V. RESEARCH

New Approaches to the Use of Twins in Biomedical Research

WALTER E. NANCE, M.D., PH.D., *Professor of Human Genetics, Pediatrics, and Medicine*

Human geneticists are often accused of being preoccupied with exotic syndromes that are of marginal relevance to the general population. Brilliant success has been achieved during the past two decades in defining the nature and function of the genetic material, the molecular pathology of a large number of metabolic diseases, the phenotype of more than 2,000 Mendelian traits, and more recently the chromosomal location of a rapidly expanding number of human gene pairs. In contrast, relatively little progress has been made in the genetic analysis of quantitative traits such as blood pressure, serum cholesterol, intelligence quotient (IQ), skin color, height, birth weight, or glucose tolerance. Traits of this type are not only of interest to society, but may also relate significantly to a variety of common diseases. With almost every continuously distributed quantitative trait, single gene defects have been identified which can profoundly alter the phenotype. For example, the single gene pairs which determine albinism and Tay-Sachs disease can profoundly alter skin color and IQ respectively. However, the causes of less extreme variation can be exceedingly complex, resulting from the cumulative effects of many gene pairs and their interactions with each other and with the environment. Nevertheless, even if the effects of individual gene pairs cannot be identified, the source of the observed variation may often be inferred from an analysis of the phenotypic correlation of relatives of various degree. Twin studies have been widely used in the past to gain insight into the inheritance of quantitative traits, and with the support of a Program Project Grant from the National Institute of Maternal and Child Health, the Department of Human Genetics at the Medical College of Virginia has become a leader in the use of twins for biomedical research.

The basic idea of a classical twin study is to compare the differences between monozygotic (MZ) and dizygotic (DZ) twins. Since MZ twins arise from a single fertilized egg, they possess identical sets of nuclear genes, and differences between them are assumed to arise from environmental causes. DZ twins, on the other hand, are genetically no more similar than siblings, sharing, on the average, only half of their genes. The extent to which the intrapair differences of DZ twins exceed those of MZ twins provides a measure of the importance of genetic factors as a cause for variation in DZ twins. DZ twins are considered to be an appropriate comparison group because they are born at the same time and are assumed to share the same environmental similarities as MZ twins. Classical twin studies remain a very useful method for estimating the relative importance of genetic factors as a cause for variation in complex physical, physiological, or psychological traits. We have made extensive use of this approach to investigate the genetic control of bone mineral content,[1] dental and cephalometric variables,[2,3,4] electrocardiographic variables,[5] amino acid metabolism,[6] dermatoglyphic

This is paper #35 from the Department of Human Genetics of the Medical College of Virginia and was supported in part by USPHS Grant P01HD10291-01.

Correspondence and reprint requests to Dr. Walter E. Nance, Box 33, Medical College of Virginia, Richmond, Virginia 23298.

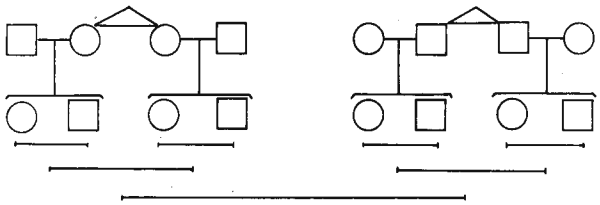

Fig 1—Pedigrees of families of monozygotic twins. Lines below figures suggest how variation among the offspring may be partitioned into among half-sibship, between sibship within half-sibship, and within sibship components. Squares indicate males and circles, females.

variables,[7] and a variety of biochemical, physiological, and psychological traits.[8,9,10] However, there are a number of limitations in the classical twin model. The method does not permit the recognition of maternal effects or an incisive resolution of the genetic effects, and the assumption that the environmental similarities of identical and fraternal twins are equivalent may be open to question for some traits. Also, in order to generalize the results, we must be willing to consider twins a random sample of the genotypes in the general population. This is probably a reasonable assumption for MZ twins; however, many genetic and environmental factors are known which profoundly influence the incidence of DZ twins and might, therefore, prevent direct extrapolation of the results of a twin study to the general population.[11] Finally, both fraternal and identical twins may be exposed to unique intrauterine environmental effects which need to be better understood in order to evaluate their possible significance as a source of bias in classical twin studies.[9]

In order to circumvent these problems, we have developed a powerful new model for the analysis of quantitative inheritance in man which exploits the unique relationships that exist within the families of identical twins.[12] Because they have a genetically identical parent, the children of identical twins are related to each other in the same way as half-siblings (Fig 1). Genetically, the child of a twin is just as closely related to his twin aunt or uncle as he is to his twin parent. However, since the parents and their own children usually live in the same home, and are, for example, exposed to the same diet, we would expect any significant environmental similarities to make an additional contribution to the parent-offspring correlation. It is easy to see how a comparison of the correlations would permit a clear separation of genetic and environmental effects on the closely related half-sibs. Each family contains individuals who share one fourth of their genes (the half-sibs), one half of their genes (the full sibs), all of their genes (the MZ twins), and none of their genes (the spouses of the twins). These and other relationships permit the derivation of multiple linear equations in which an observed correlation, variance or covariance between family members is expressed as a linear combination of several unknown genetic and environmental variance components that we wish to estimate. Since the model yields more equations than unknowns, we can solve the system of equations and obtain the best fitting estimates of the unknown parameters. The calculations are quite complicated, particularly since we must allow for the fact that our observed correlations, variances, and covariances may not be independent of one another because they have been derived from individuals who are interrelated in a complex manner. However, the use of a high-speed computer makes solution of the equations technically feasible.

The entire process of parameters estimation can be summarized in matrix notation by the single equation, $\hat{G} = (M'V^{-1}M)^{-1} M' V^{-1}C$. G represents the vector of unknown genetic variance components we wish to estimate; M represents the matrix of coefficients derived from Table 1; C represents the vector of the observed mean squares; and V represents the variance/covariance matrix of the observed mean squares. Most physicians would probably find a detailed exposition of the mathematical analysis to be tedious and uninformative; however, the essence of the analysis is simple and intuitive, depending

TABLE 1
Genetic Interpretation of Variance Components from Offspring Analysis of Variance

	Constituent genetic and environmental subcomponents						
Variance Component	V_A	V_D	V_{AA}	V_M	V_{EH}	V_{ES}	V_{EW}
Among half-sibship, male twins	1/4	0	1/16	0	1	0	0
Between sibships, male twins	1/4	1/4	3/16	1	0	1	0
With sibships	1/2	3/4	3/4	0	0	0	1
Between sibships, female twins	1/4	1/4	3/16	0	0	1	0
Among half-sibships, female twins	1/4	0	1/16	1	1	0	0

TABLE 2
Analysis of the Birth Weight of 254 Offspring of 46 Pairs of Identical Twins

Parameter	Estimate ± Standard Error	Proportion of total variance (%)
V_{AA}	0.014 ± 0.037	6.4
V_M	0.075 ± 0.030	34.1
V_{ES}	0.049 ± 0.020	22.2
V_{EW}	0.082 ± 0.028	37.3

as it does upon the concept developed in high school algebra, that if you have as many independent equations as you have unknowns, a solution can be obtained.

A unique feature of the model is that it permits the detection and estimation of genetically determined maternal effects. If intrauterine or postnatal maternal effects influence a trait to a significant degree, we would expect the offspring of identical female twins to be more similar than the offspring of male twins since the latter are born to genetically unrelated mothers. As suggested by Figure 1, the total variability or variance among the offspring may be divided by a statistical procedure known as a "nested analysis of variance" into within sibship, between sibship within half-sibship, and among half-sibship components. Each of these components may be expressed in terms of a series of constituent genetic and environmental subcomponents (Table 1). In this formulation, V_A refers to the additive component of the genetic variance. Additive genetic effects are the ones responsible for the resemblance between parents and their offspring. The among half-sibship component of variance is equivalent to the covariance of half-sibs and since half-sibs share one fourth of their genes, this component includes one fourth of the total variation attributable to additive effects, the remainder being distributed as shown in Table 1. V_{AA} refers to additive genetic effects that result from the interaction of genes at separate loci. The term V_D refers to genetic effects that show dominance and arise from interactions between the two members of a gene pair (alleles). Dominance effects can contribute to the similarity only of individuals who are related to each other through both parents. For this reason, note that no dominance effects appear in the among half-sibship component. V_{EH}, V_{ES}, and V_{EW} refer to environmental effects acting among half-sibships, between sibships within half-sibships, and within sibships respectively. Finally, maternal effects are designated by V_M. Since they contribute to the similarity of the half-sib offspring of female twins, V_M appears in the among half-sibship component for female twins. In the case of male twins, on the other hand, the maternal effects arising from the genetically unrelated wives contribute to variation between sibships within each half-sibship. The five relationships shown in Table 1 provide five of the equations which can be used in the overall analysis described previously. If the analysis is confined to these relationships, it becomes possible to exploit the unique genetic relationship of monozygotic twins through observations of their offspring without incurring any of the methodologic liabilities that might be associated with the inclusion of data from the twins themselves.

Table 2 shows the results of an analysis of the birth weight of 254 children of 46 twin pairs. The data were collected by interviews and questionnaires, and prior to the analysis, the birth weights were adjusted for gestational age at the time of delivery. In all genetic models that fit the data well, there was evidence that genetically determined maternal effects made a highly significant contribution to the overall variation in birth weight. In fact, our results suggest that at least one third of the total variation in birth weight is actually fixed or determined prior to the time of conception by the genotype of the mother! Since birth weight is highly correlated with fetal survival, these findings raise the possibility that perhaps we should look more closely at the mothers of high and low birth weight infants; in future extensions of the present work, we would like to repeat the analysis

TABLE 3
Distribution of Twins by Age, Sex, and Zygosity

Type	Age in years 0–10	10–20	20	Total
MONOZYGOTIC				
Male-Male	15	11	6	32
Female-Female	14	3	10	27
Total	29	14	16	59
DIZYGOTIC				
Male-Male	9	1	0	10
Female-Female	9	1	3	13
Male-Female	21	2	2	25
Total	39	4	5	48
GRAND TOTAL	68	18	21	107

TABLE 4
Distribution of Newborn Twins by Race and Placenta Type

Placenta type	Black		White		Total	
	MZ	DZ	MZ	DZ	MZ	DZ
TWINS						
Monochorionic monoamnionic	1	0	0	0	1	0
Monochorionic diamnionic	6	0	2	0	8	0
Dichorionic, fused	1	4	0	4	1	8
Dichorionic, separate	1	16	0	5	1	21
TRIPLETS						
Monochorionic triamnionic	1	0	0	0	1	0
Trichorionic	0	1	0	0	0	1
TOTAL	10	21	2	9	12	30

after dividing the families into high, low, and intermediate birth weight categories. We are currently trying to identify additional fertile adult MZ twins who would be suitable for inclusion in our MZ half-sib studies. The project involves the collection of a large amount of medical and genetic data from the families during an out-patient visit, and the participants are informed of any abnormalities that are detected in any of the blood, urine, or physiologic screening tests.*

Biology of Twinning

Since the new program of twin research was initiated at MCV, 107 twin pairs have been evaluated

* Twins may be referred to the study by calling the Project Coordinator, Phyllis Winter, at 804/770-4645.

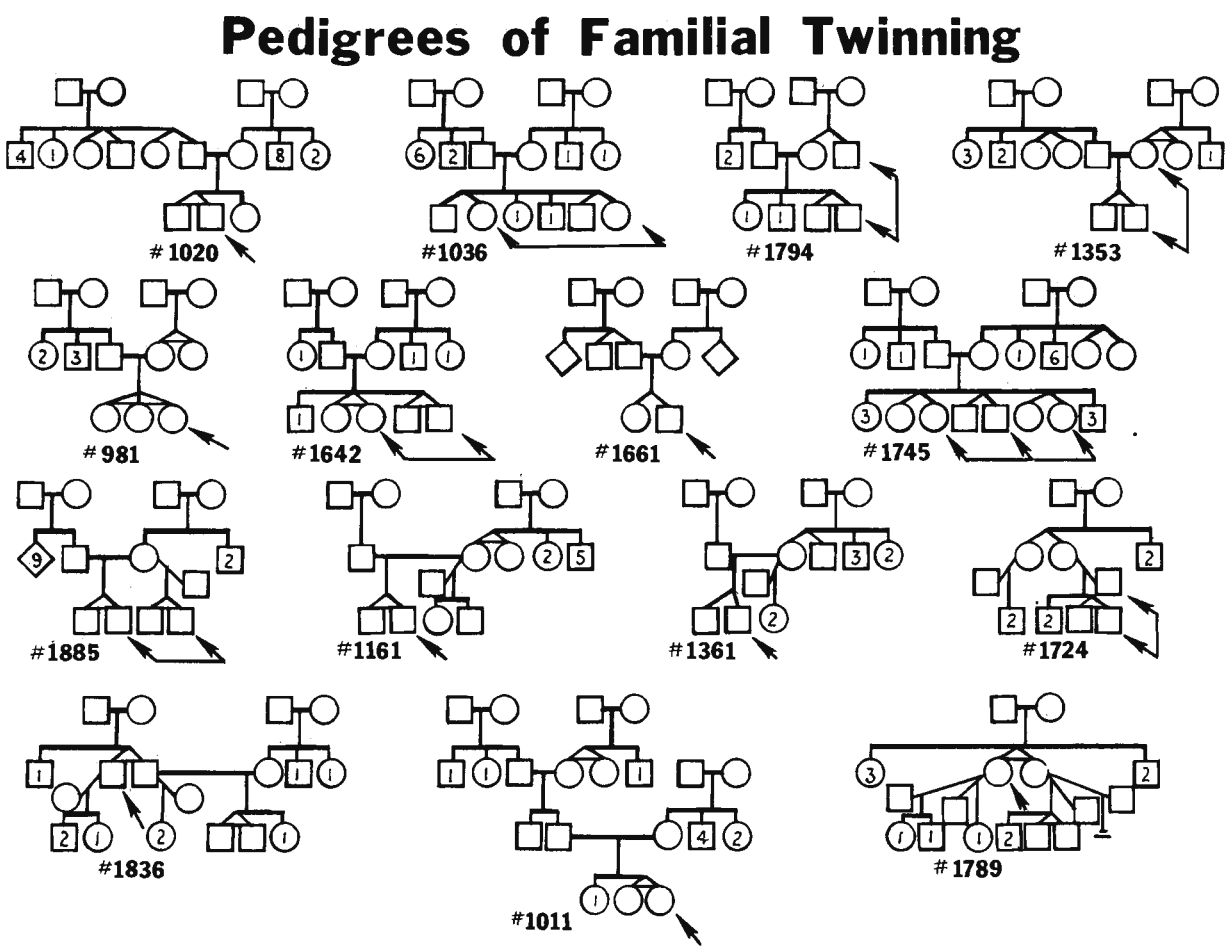

Fig 2—Pedigrees of 15 families showing a history of twinning in close relatives.

and enrolled in the Twin Panel (Table 3). The sample includes 40 pairs of newborn twins and two sets of triplets ascertained from the MCV Obstetrical Service. Systematic placental examinations have been performed in these twins (Table 4). It is now clear that monozygotic twins may have either two separate placentas or a single placenta. The former are all dichorionic while the latter include dichorionic pairs and monochorionic twins that may either be diamnionic or rarely monoamnionic. These differences in placentation are thought to reflect differences in the stage of embryonic life at which the twinning process occurred. One goal of the newborn twin study is to determine whether these striking placental differences are reflected in phenotypic differences between the twins. Preliminary evidence suggests that the intrapair differences in cord blood cholesterol in dichorionic twins are more than five times as great as those observed in monochorionic pairs.[9]

The twins studied to date have included 15 families in which there was a history of twinning in a close relative (Fig 2). A genetic predisposition to dizygotic twinning has long been recognized, presumably mediated by neuroendocrine differences in some women. For MZ twins, however, it is much less clear whether or not the occasional occurrence of familial aggregation indicates a genetic effect, and the cases shown (see Fig 2) do little to resolve this uncertainty. In family #1885, the birth of two sets of MZ twins to a woman by different fathers strongly suggests a maternal influence, whereas family #1836, in which MZ twins were born to a member of a male MZ pair, suggests patrilineal inheritance. Finally, in family #1642, placental examinations were performed on both twin pairs, revealing monoamnionic placentation in one and diamnionic monochorionic placentation in the other—a finding which suggests that familial occurrence is not confined to twins of a single placenta type.

Summary

The MCV Twin Panel was begun in 1976 and includes twins and higher order multiple births of all ages. Twin studies offer many opportunities for innovative research, and it is intended that the Panel be a research resource for clinical investigators at the Medical Center.

REFERENCES

1. Smith DM, Nance WE, Kang KW, et al: Genetic factors in determining bone mass. *J Clin Invest* 52:2800–2808, 1973.

2. Potter RH, Nance WE: A twin study of dental dimension. I. Discordance asymmetry and mirror imagery. *Am J Phys Anthropol* 44:391–396, 1976.

3. Nakata M, Yu PL, Davis B, et al: Genetic determinants of craniofacial morphology: a twin study. *Ann Hum Genet* 37:431–443, 1974.

4. Nakata M, Yu PL, Nance WE: Multivariate analysis of craniofacial measurements in twin and family data. *Am J Phys Anthropol* 41:423–430, 1974.

5. Bingle GJ: *The Inheritance of Electrocardiographic Variables in Man: A Comparison of Twin and Half-Sib Models,* thesis. Indiana University, Indianapolis, 1974.

6. Paul TD: *Analysis of the Inheritance of Normal Human Serum Amino Acid Levels by the Twin Study Method,* thesis. Indiana University, Indianapolis, 1975.

7. Nance WE, Nakata M, Paul TD, et al: The use of twin studies in the analysis of phenotypic traits in man, in Janerich DT, Skalko RG, Porter IH (eds): *Congenital Malformations: New Directions in Research.* New York, Academy Press, 1974, pp 23–49.

8. Campbell JB, Nance WE, Black K: An analysis of Bayley scores in 96 18-month-old twins, abstract. *Behav Genet* 5:90–91, 1975.

9. Corey LA, Kang KW, Christian JC, et al: Effects of chorion type on variation in cord blood cholesterol of monozygotic twins. *Am J Hum Genet* 28:433–441, 1976.

10. McCune SA, Yu PL, Nance WE: A genetic study of erythrocyte tRNA synthetase activity in man. *Acta Genet Med Gemellol* 26:21–27, 1977.

11. Nance WE: Note on the analysis of twin data, letter to the editor. *Am J Hum Genet* 28:297–299, 1976.

12. Nance WE, Corey LA: Genetic models for the analysis of data from the families of identical twins. *Genetics* 83:811–826, 1976.