THE NATIONAL HEART AND LUNG INSTITUTE TWIN STUDY OF CARDIOVASCULAR DISEASE RISK FACTORS: ORGANIZATION AND METHODOLOGY

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The National Heart and Lung Institute undertook a twin study on the etiology of coronary heart disease and genetic relations underlying differential levels of coronary risk. Between 1969 and 1974, 250 MZ and 264 DZ male twin pairs aged 42-56 were examined. The examination featured a medical and family history, a dietary interview, ECG, blood pressure, weight and height measurement, a variety of blood chemistry tests, including complete lipoprotein analyses, and lung function tests. Zygosity was determined through 22 red cell antigens. A detailed interview dealing with the twins' relationships to each other was also obtained. Each of the quantitative variables was tested for the presence of genetic variance using the method of Christian. This method first tests the equality of the total variances of MZ and DZ twins by a two-tailed F' test. When the hypothesis of equality of these two variances is rejected, use of the among-component estimate of genetic variance is indicated. The current report discusses the organization and methodology of the study while accompanying reports focus on the genetic variance in blood lipids, blood pressure, and coronary-prone behavior patterns.

Many prospective epidemiologic studies conducted during the past twenty years have firmly established the association between several personal and physiological characteristics and the occurrence of coronary heart disease. Elevated blood pressure, increased plasma cholesterol and cigarette smoking are among the key factors which have been related to an increased risk of premature heart disease. Several intervention studies are underway at the National Heart and Lung Institute to determine whether modification of these factors once they are established will result in a reduction of the frequency of clinical heart disease. Other NHLI programs are concerned with studies of the natural history of the risk factors themselves, including their genetic and familial patterns. The purpose of the present report is to give a brief description of the methods used in one of these studies, the NHLI Twin Study, the plan of which was presented at the First International Symposium on Twin Studies held here 5 years ago (Feinleib et al. 1970).

This morning we have heard several reaffirmations of Sir Francis Galton's belief of the potential usefulness of comparing MZ twins with DZ twins to estimate the relative contributions of genetic factors vs. environmental factors in determining the variability of human traits. Although this Congress attests to the importance of twin studies in modern research, it is safe to say that twin studies have not yet achieved the full potential envisioned by Galton. This has been due to a variety of practical, methodologic and theoretical difficulties which have included the difficulty of obtaining large representative samples of twins, ascertaining their zygosity accurately, performing measurements

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in accurate and comparable fashion in all twins, and developing adequate statistical and genetic models for analyzing the resulting data. We believe that the current twin studies at the NHLI have overcome many of these problems only to reveal other areas of difficulty which were assumed to be of little importance by previous investigators.

The NHLI Twin Study is a standardized multicenter investigation of adult white male twins accertained through the NAS-NRC Twin Registry (Jablon et al, 1967). All of the twins were born between 1917 and 1927 in the contiguous United States. They served in the armed forces either during World War II or during the Korean conflict and were between 42 and 56 years of age when examined as part of the NHLI Twin Study. The NAS-NRC Twin Registry contains over 16,000 pairs of such twins of whom about 7,000 responded to initial mail questionnaires during the early 1960's. Invitations were sent to 1,098 twin pairs; 189 individuals were either dead, unable to reply or had their letters returned as undeliverable, resulting in 980 twin sets in which both members were contacted. Of these 1,960 individuals, 24% refused to be examined and another 9% did not reply to any of the mail solicitations. Both members of 514 twin sets eventually participated in the study. The zygosity of the twins was determined through blood antigen analyses (A, A_1, B, M, N, S, s, P, C, C^w, D, D^u, E, c, e, K, $k_p{}^a$, $K_p{}^b$, k, $L_e{}^a$, $F_y{}^a$, $J_k{}^a$) which confirmed the twins' opinions of their zygosity in virtually every case.

The twins were drawn from three geographical areas in the United States and were examined at five examination centers, selected so that no twin had to travel more than 200 miles to be examined. Table 1 shows the dates of operation and number of pairs examined at each center. Pretesting of all forms and procedures was done in the Washington, D.C. area on a sample of 72 twin pairs obtained through a registry compiled by the National Eye Institute. The pretest sample was not included in the final series.

Table 1

Study center	Dates of examination	Number of twin sets		
		MZ	DZ	Total
Framingham	7/69- 5/70	55	50	105
Indiana	12/71- 1/73	68	73	141
San Francisco	7/70- 1/71	16	38	54
Davis	11/70- 9/71 4/73- 8/73	33	45	78
Los Angeles	11/71-11/72	78	58	136
All	7/69- 8/73	250	264	514

Table 2

Data source	Summary of contents
Personal and family history	Occupational history, twins' opinion of CVD morbidity and mortality for first degree relatives
Twin history	Twins' opinions of their relationships to each other
Diet history	Twins' evaluation of how often they eat various foods
Medical history	Medication used, smoking history of respiratory symptoms and vascular disease
Physical examination	Emphasis on respiratory system, heart, peripheral vessels, neurology
Electrocardiograph	Based on 13 lead ECG
Numerical data	Anthropometry, blood pressure, lung function, glucose tolerance
Laboratory data	14 blood chemistries and red blood cell antigens
Blood lipids	HDL, LDL, VLDL, Fredrickson classification

Table 3. *Analysis of twin data*

	MZ		DZ	
		DF		DF
Means	$\bar{X}_{MZ}$	M	$\bar{X}_{DZ}$	D
Anova				
Among mean squares	A_{MZ}	M-1	A_{DZ}	D-1
Within mean squares	W_{MZ}	M	W_{DZ}	D

Each of the twins came to the examination center in the morning after an overnight fast of at least 14 hours. An effort was made to have both twins come in on the same day, in order to minimize secular differences, but they were examined by separate physicians to minimize observer biases. Standard forms modeled after those used at the Framingham Heart Study were used at each of the centers, and each examining team was instructed and checked periodically for uniform adherence to the study protocol. As shown in Table 2, the examinations included a detailed family, medical, twin, and dietary history on each individual. Physical examination of the

head, neck, chest and limbs was conducted and pulmonary function tests and a 13 lead electrocardiogram were done. Blood chemistries were performed at the local centers but the blood lipids were done at special study laboratories which participate in the CDC standardization program and which exchanged specimens to maintain standardization between the centers. After the forms were completed and checked at the local centers they were sent to the Epidemiology Branch, NHLI in Bethesda, Maryland for transfer to magnetic tape and statistical analysis.

Table 3 lists the summary statistics that were utilized in the analysis of the NHLI Twin Study data. The means for MZ and DZ twins were compared for each variable and significant differences interpreted as indicative of zygosity effects which might cast doubt on subsequent analyses of variance. Variance among centers was tested for significant differences that might indicate systematic measurement differences among centers or true geographical effects. Among-center differences were not adjusted because they may reflect true subpopulation differences, and their removal would give a biased view of total population variation.

As previous speakers have indicated, there have been several methods suggested for estimating genetic variance of quantitative traits in twins. Most previous investigators have relied upon the results of an F test with the within DZ mean square as the numerator and the within MZ mean square as the denominator as an indication of genetic influence on a quantiative trait.

A method described by Christian et al. (1974) has been adopted for the analysis of the NHLI Twin Study data. This method first tests the equality of the total variances of MZ and DZ twins by a two-tailed F' test. Inequality of total variances is interpreted as an indication that environmental components of variance for MZ and DZ twins are not equal, although other hypotheses are also tenable. Table 4 gives the expected values of the among- and within-twin-pair mean squares using the notation presented by Haseman and Elston (1970). These mean squares can be manipulated in a variety of ways to give several different estimates of genetic variance as shown in Table 5. Estimates based on

Table 4. *The twin model components of variance for expected mean squares*

$$E(A_{MZ}) = 2\sigma^2_a + 2\sigma^2_d + 2\sigma^2_i + \sigma^2_{EMZ} + 4\sigma_{ge} + C_{MZ}$$

$$E(W_{MZ}) = \sigma^2_{EMZ} - C_{MZ}$$

$$E(A_{DZ}) = 3/2\,\sigma^2_a + 5/4\,\sigma^2_d + (1+f)\sigma^2_i + \sigma^2_{EDZ} + 4\sigma_{ge} + C_{DZ}$$

$$E(W_{DZ}) = 1/2\,\sigma^2_a + 3/4\,\sigma^2_d + (1-f)\sigma^2_i + \sigma^2_{EDZ} - C_{DZ}$$

differences of the within- or among-mean squares will be useful if the environmental components of variance of MZ and DZ twins are equal as well as the respective environmental covariances. If there is doubt that the total environmental variances of the two zygosity groups are equal, then these estimates may contain serious biases. Kempthorne and Osborne (1961) have discussed environmental factors that could affect MZ and DZ twins differently before and after birth. An estimate suggested by Christian using all four mean squares is not influenced by total environmental variance although it does contain the two environmental covariances. We call this estimate the among-component estimate of genetic variance and its significance may be tested by an F' test. The power of the test of significance of the among-component estimate is generally less than that of the within-pair estimate and would require larger numbers of twin pairs to yield statistical significance.

As can be seen in Table 5, all three estimates of genetic variance are made up of fractions of additive, dominance and epistatic genetic variance. Therefore, the test for the presence of genetic variance can be precisely stated as $H_o : \sigma^2_A = 0$, $\sigma^2_d = 0$, and $\sigma^2_i = 0$. When the null hypothesis is rejected, no inference as to the source of the genetic variance, be it additive, dominance or epistatic, is possible. However, using the commonly made assumption that dominance and epistatic variance are negligible,

Table 5

Significance test	Linear combination of mean squares and their expected value
$F = \frac{W_{DZ}}{W_{MZ}}$	$\hat{G}_{WT} = W_{DZ} - W_{MZ}$
	$E(\hat{G}_{WT}) = \frac{1}{2}\sigma^2_a + \frac{3}{4}\sigma^2_d + (1-f)\sigma^2_i + \sigma^2_{EDZ} - \sigma^2_{EMZ} - C_{DZ} + C_{MZ}$
$F = \frac{A_{MZ}}{A_{DZ}}$	$\hat{G}_{AT} = A_{MZ} - A_{DZ}$
	$E(\hat{G}_{AT}) = \frac{1}{2}\sigma^2_a + \frac{3}{4}\sigma^2_d + (1-f)\sigma^2_i + \sigma^2_{EMZ} - \sigma^2_{EDZ} + C_{MZ} - C_{DZ}$
$F' = \frac{A_{MZ} + W_{DZ}}{A_{DZ} + W_{MZ}}$	$\hat{G}_{AC} = \frac{(W_{DZ} - W_{MZ}) + (A_{MZ} - A_{DZ})}{2} = \frac{A_{MZ} + W_{DZ} - (A_{DZ} + W_{MZ})}{2}$
	$E(\hat{G}_{AC}) = \frac{1}{2}\sigma^2_a + \frac{1}{3}\sigma^2_d + (1-f)\sigma^2_i + C_{MZ} - C_{DZ}$

doubling any of the estimates in Table 5 will give an estimate of total population additive genetic variance. Relating this estimated additive genetic variance to the observed total variance (pooled for MZ and DZ twins) gives an indication of the extent to which population variability of a trait results from genetic differences in the population.

The results of these analyses with regard to three major risk factors for coronary heart disease, namely blood pressure, plasma lipids and personality behavior types, will be presented in other talks at this Congress (Borhani et al. 1974, Christian et al. 1974, Rosenman et al. 1974).

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