Am J Hum Genet 28:433–441, 1976

Effects of Chorion Type on Variation in Cord Blood Cholesterol of Monozygotic Twins

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INTRODUCTION

An obvious common force affecting monozygotic (MZ) twins is their prenatal environment. Further, MZ twins are separable into two classes according to type of intrauterine development: those having a single chorion (monochorionic) and those having separate chorions (dichorionic). These differences are thought to correspond to the stage of early embryonic life at which the twinning process occurs, with dichorionic twins arising at a very early stage and monochorionic twins possibly as late as the second week of gestation [1]. Some authors have suggested subclassifying twins to compare single vs. double chorionic development [1]. However, most studies of MZ twins have either failed to recognize the dichotomy in prenatal environment or have not had adequate obstetrical data to make the subdivision [1, 2]. Since similarity in prenatal environment may be related to chorionic development, failure to consider such effects may introduce a systematic bias, especially with regard to quantitative traits measured just after birth. In particular, chemical constituents of blood may be differentially influenced by the almost inevitable occurrence of vascular anastamoses in the placentae of monochorionic and their absence in dichorionic twins [1].

In earlier studies of variation in cholesterol levels of adult twins [3] and in a subsample of the newborn twins examined in this study [4, 5], the total variance (among mean square + within mean square) of MZ twins was found to be smaller than that of dizygotic (DZ) twins. Christian et al. [6] have suggested that the study of newborn twins might be useful in determining if the discrepancy

Received March 1, 1976; revised May 20, 1976.

This research (publication no. 76-6 from the Department of Medical Genetics) was supported in part by the Indiana University Human Genetics Center (PHS GM 21054), Institutional Research Fellowship in Oral-Facial Genetics (PHS DE 00007), and a grant from the John A. Hartford Foundation, Inc.

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between MZ and DZ twins is due to pre- or postnatal factors. The present study was designed to determine if placental type influences cholesterol level or its variability in newborn MZ twins and to present a statistical method for comparing MZ twins of different placental types.

MATERIALS AND METHODS

Samples of cord blood were collected from 30 pairs of monochorionic and 22 pairs of dichorionic newborn MZ twins. The number of chorions was established by gross and microscopic examination of fetal membranes. Zygosity was confirmed by typing for nine polymorphic blood group systems. The monochorionic class was not further subdivided by amnion type because of the small number within the monoamniotic subgroup. Female monochorionic pairs outnumbered male monochorionic pairs by approximately 3 to 1 (22 vs. 8 pairs); there was an equal number of dichorionic male and female pairs (11 pairs).

Cholesterol was saponified and extracted from cord plasma in alcoholic potassium hydroxide and hexane. The cholesterol phase was concentrated under nitrogen and then prepared for spectrophotometric measurement with modified Lieberman-Burchard reagent [7]. Cholesterol samples were compared with distilled water at 620 nm and absorbance with a cholesterol standard (Supelco Inc., Bellefonte, Pa.). Values are expressed as milligrams cholesterol per 100 ml plasma.

Statistical Methods

The general linear model for the analysis of twin data [2, 8] can be applied separately to monochorionic and dichorionic twins. In this paper, the assumed statistical model is intended specifically for quantitative characters which may be subject to prenatal conditioning (i.e., cord blood cholesterol). Let us represent the observed values for members of the ith twin pair of a given sex-chorion classification as $X_{i1} = \mu + g_i + e_{i1}$, $X_{i2} = \mu + g_i + e_{i2}$, $i = 1, \ldots, n$, where μ is the population mean, g_i is the effect of the ith genotype, and e_{i1} and e_{i2} are the effects of prenatal environment on each member of the pair. The g_i's, e_{i1}'s, and e_{i2}'s are assumed to be random variables normally distributed about zero means so that $E(g_i^2) = \sigma_g^2$, $E(e_{ij}^2) = \sigma_e^2$, $E(g_i e_{ij}) = \sigma_{ge}$, and $E(e_{i1}e_{i2}) = \sigma_{ee}$.

Analyses of variance with expected mean squares are given for monochorionic and dichorionic MZ twins in table 1. Because of the dissimilarity in prenatal circumstances, corresponding environmental components (σ_e^2, σ_{ee}, and σ_{ge}) in the two sets of expected mean squares may best be regarded as possibly heterogeneous; in the table, subscripts 1 and 2 denote monochorionic and dichorionic environmental components, respectively. In contrast, the type of chorion development is assumed to have no effect on the sampling of genetic effects, and the genetic variance component (σ_g^2) is considered to be the same parameter in both analyses. Since genetic or racial factors are not known to influence the relative frequencies of monochorionic and dichorionic MZ twins, it seems reasonable to assume that the total variance attributable to differences in nuclear genes in the two classes are equal. Variation due to genotype $\times$ environment interaction has not been partitioned from total environmental variation. For this reason, environmental components should best be thought of as containing variation attributable to $g \times e$ interaction should it exist as well as other environmental sources.

Based on the expectations in table 1, individual components of variance and covariance cannot be estimated from the observed mean squares. However, certain linear functions of the components can be estimated, and some of these may be of interest. For instance, $E(A_k + W_k) = 2\sigma_{ek}^2 + 4\sigma_{gek} + 2\sigma_g^2$, $E(A_k - W_k) = 2\sigma_{eek} + 4\sigma_{gek} + 2\sigma_g^2$, $k = 1, 2$. Thus, $E(A_2 - A_1 + W_2 - W_1) = 2(\sigma_{e_2}^2 - \sigma_{e_1}^2) + 4(\sigma_{ge_2} - \sigma_{ge_1})$, and $E(W_2 - W_1) = \sigma_{e_2}^2 - \sigma_{e_1}^2 + \sigma_{ee_1} - \sigma_{ee_2}$. Furthermore, if the twin means and differences of each

TABLE 1

ANALYSES OF VARIANCE FOR MONOCHORIONIC AND DICHORIONIC MZ TWINS

SOURCE OF VARIATION	df	MONOCHORIONIC		DICHORIONIC	
		Mean Square	Expected Mean Square	Mean Square	Expected Mean Square
Among pairs	$n-1$	A_1	$\sigma_{e_1}^2 + \sigma_{ee_1} + 2(\sigma_g^2 + 2\sigma_{ge_1})$	A_2	$\sigma_{e_2}^2 + \sigma_{ee_2} + 2(\sigma_g^2 + 2\sigma_{ge_2})$
Within pairs	n	W_1	$\sigma_{e_1}^2 - \sigma_{ee_1}$	W_2	$\sigma_{e_2}^2 - \sigma_{ee_2}$

* Components of expected mean squares for the k chorion type are defined as follows: σ_{ek}^2 is the variance of prenatal effects on individuals of twin pairs, σ_{eek} is the intra-pair covariance between prenatal effects, σ_{gek} is the covariance between the effects of fetal genotype and prenatal environment, and σ_g^2 is the variance due to genetic differences among twin pairs, where $k = 1, 2$ for monochorionic or dichorionic twins, respectively.

chorion group are normally distributed, then two-tailed F tests can be performed to judge the equality of pertinent linear functions of components. Suppose, for example, that $A_2 > A_1$ and $W_2 > W_1$, then $F_A = A_2/A_1$ tests $\sigma_{e_1}^2 + \sigma_{ee_1} + 4\sigma_{ge_1} = \sigma_{e_2}^2 + \sigma_{ee_2} + 4\sigma_{ge_2}$, $F_W = W_2/W_1$ tests $\sigma_{e_1}^2 - \sigma_{ee_1} = \sigma_{e_2}^2 - \sigma_{ee_2}^2$, $F'_{A+W} = (A_2 + W_2)/(A_1 + W_1)$ tests $\sigma_{e_2}^2 + 2\sigma_{ge_2} = \sigma_{e_1}^2 + 2\sigma_{ge_1}$, and $F'_{A-W} = (A_1 - W_1)/(A_2 - W_2)$ tests $\sigma_{ee_1} + 2_{ge_1} = \sigma_{ee_2} + 2\sigma_{ge_2}$. Degrees of freedom for complex F tests can be calculated by the method of Satterthwaite [9, 10]. Since there has been some question about the validity of the approximate F' test when some mean squares are subtracted from others [9–11], the hypothesis estimated by F'_{A-W} may be tested by $F' = (A_1 + W_2)/(A_2 + W_1)$.

A linear measure of discordance for MZ twins is given by the mean intra-pair range $\bar{R} = \Sigma|X_{i1} - X_{i2}|/n$ which can be expressed in model terms as $\Sigma|e_{i1} - e_{i2}|/n$. If the model assumptions are correct (i.e., if e_{i1}, e_{i2} have a bivariate normal distribution with a mean equal to zero, variance equal to σ_e^2, and a covariance σ_{ee}), then $\bar{R}$ can be shown to have an expected value of $2(\sigma_e^2 - \sigma_{ee})^{1/2}/\pi^{1/2}$ and a variance $2(\sigma_e^2 - \sigma_{ee})(1 - 2/\pi)/n$. Consequently, 0.886 $\bar{R}$ provides an unbiased estimate of $(\sigma_e^2 - \sigma_{ee})^{1/2}$ and $s_{\bar{R}}^2 = 0.727\ W/n$. In fact, a comparison of the two estimates of $(\sigma_e^2 - \sigma_{ee})^{1/2}$ obtained from $\bar{R}$ and W might shed some light on the distributional characteristics of the data examined within each chorion class. If the distribution is bimodal, it might be expected that the estimate obtained using W would be greater than that obtained using $\bar{R}$. Again denoting terms for monochorionic and dichorionic classes by subscripts 1 and 2, respectively, we suggest that $t' = |\bar{R}_1 - \bar{R}_2|/(s_{\bar{R}_1}^2 + s_{\bar{R}_2}^2)^{1/2}$ can be used to test the equality of $(\sigma_{e_1}^2 - \sigma_{ee_1})^{1/2}$ and $(\sigma_{e_2}^2 - \sigma_{ee_2})^{1/2}$.

RESULTS

The data of each sex chorion class were first examined for departures from normality [12]. In general, the results obtained justify the use of standard statistical procedures (t tests and F tests) in comparing means or mean squares of the different classes.

The cord blood cholesterol means for MZ twins of the four sex chorion classes in table 2 indicate that the average cholesterol content of females is higher than

TABLE 2

MEAN CHOLESTEROL LEVELS (MG/100 ML) WITH STANDARD ERRORS IN CORD BLOOD OF MONOCHORIONIC AND DICHORIONIC MZ TWINS

SEX	MONOCHORIONIC		DICHORIONIC		COMBINED			
	No. of Pairs	$\bar{X}$	No. of Pairs	$\bar{X}$	No. of Pairs	$\bar{X}$	g_1*	g_2
Female	22	86.0 ± 5.2	11	80.9 ± 7.0	33	84.3 ± 4.2	−0.03	−0.59
Male	8	69.6 ± 9.1	11	69.6 ± 8.2	19	69.6 ± 6.1	0.19	0.24
Sex differences	...	16.4		11.3		14.7		
		$P < .06$†		$P < .15$		$P < .03$		

* g_1 and g_2 denote estimates of skewness and kurtoses, respectively, for male and female twin pair populations.
† In tests of significance, the variance of a single pair mean was estimated as $s^2 = \Sigma vA/2\Sigma v$, where A denotes an among-pair mean square and v the corresponding degrees of freedom (see table 3).

males. This is evidenced by the statistical significance of the overall difference in cholesterol level between males and females, as well as by the consistency in sign and magnitude of sex differences for each type of placental development. Within sexes, chorion type apparently had little or no effect on the cholesterol level.

Results from the analyses of cholesterol data are presented in table 3. Despite

TABLE 3

ANALYSES OF VARIANCE OF CORD BLOOD CHOLESTEROL IN MONOCHORIONIC AND DICHORIONIC MZ TWINS

Sex	Source of Variation	Monochorionic df	Monochorionic Mean Square	Dichorionic df	Dichorionic Mean Square	F_A	F_W	$F'_{(A+W)}$	$F'_{(A-W)}$
Female	Among pairs	21	1,213	10	1,077	1.1	5.2*	1.5	16.7
	Within pairs	22	194	11	1,016	...	...	...	...
Male	Among pairs	7	1,345	10	1,483	1.1	8.0*	1.6	2.1
	Within pairs	8	111	11	887	...	...	...	...
Pooled over sexes	Among pairs	28	1,246	20	1,280	1.0	5.5*	1.6	3.3
	Within pairs	30	172	22	951	...	...	...	...

* Denotes statistical significance at the .01 level of probability.

the mean sex differences observed within each chorion class, corresponding estimates of variability determined from male and female pairs were not heterogeneous; consequently, pooled results have also been given for each type of placental development.

Two evincive trends are reflected in the mean squares and simple F tests (F_A and F_W) of table 3. First, the among-pair mean squares of the four sex chorion classes show homogeneity, and in fact, the pooled estimates of variability among pairs of each chorion type are remarkably similar. Second, within-pair mean squares of the two placental classes are heterogeneous. Further, in comparisons between classes, the observed variation within dichorionic pairs is at least five times greater than the variation within monochorionic pairs. Thus, while sex and type of placental development seem to have little effect on variation in cholesterol level *among* MZ pairs, the prenatal environment of dichorionic twins apparently gives rise to greater *within*-pair variation than that of monochorionic pairs.

Table 4 gives the mean intra-pair range in cord blood cholesterol level observed within each sex chorion class and pooled results for each chorion type. As seen in table 4, intra-pair ranges of the dichorionic class exceed those of the monochorionic class by the same magnitude for each sex, and the overall difference between chorion classes approaches significance at the .05 level of probability. These results also suggest that dichorionic MZ twins are subject to greater average discordance in cholesterol level than are monochorionic twins.

TABLE 4

MEAN INTRA-PAIR RANGE ($\bar{R}$) IN CORD BLOOD CHOLESTEROL (MG/100 ML) OF MONOCHORIONIC AND DICHORIONIC MZ TWINS

SEX	MONOCHORIONIC		DICHORIONIC		
	No. of Pairs	$\bar{R}_1$	No. of Pairs	$\bar{R}_2$	$\bar{R}_1 - \bar{R}_2$
Female	22	14.7	11	24.9	10.2 $P < .12$
Male	8	12.3	11	22.5	10.2 $P < .14$
Combined	30	14.0	22	23.7	9.7 $P < .06$

NOTE.—In tests of significance, the variance of $\bar{R}_j$ was estimated as $s_{\bar{R}}^2 = 0.727\ W_j/n$ where W_j denotes the pooled within-pair mean square for the corresponding chorion class.

DISCUSSION

In this study, the mean cord blood cholesterol level of female MZ twins was significantly higher than that of males when data was combined over chorion types ($P < .05$). While similar findings have been reported from several studies of newborns [13, 14], there is conflicting evidence on the existence of a sex difference in blood cholesterol for other age groups [15, 16]. Consequently, it is difficult to say how long the higher cholesterol level of newborn females persists. Further work needs to be done to determine the etiology and transiency of neonatal sex differences in blood cholesterol.

Based on the observed measures of within-pair variation for MZ twins (W and $\bar{R}$), it appears that members of dichorionic pairs tend to be more discordant in blood cholesterol content than members of monochorionic pairs. In a statistical sense, this indicates that the effects of prenatal environment associated with the placental types either have a different variance ($\sigma_{e_2}^2 < \sigma_{e_1}^2$), a different covariance ($\sigma_{ee_1} < \sigma_{ee_2}$), or both.

At this point, it is informative to express the total prenatal effect of the ith mother with a particular chorion type on the cholesterol level of her twin pair as the sum of two parts (i.e., $E_i = e_{i_1} + e_{i_2}$). Using this symbolism, the mother to mother variation of prenatal effects in the kth chorion type can be written as $\sigma_{E_k}^2 = 2\sigma_{e_k}^2 + 2\sigma_{ee_k}$ where $k = 1, 2$. Since the development of single vs. double chorions appears to be a random phenomenon in MZ twinning [1], it seems unlikely that placental type would affect the sampling variance of E_i. This is indirectly supported by the observed data in that mean cholesterol values as well as the among-pair mean squares are quite similar for monochorionic and dichorionic twins. For these reasons, the interpretation of further results will be based on the assumption that mother to mother variation in prenatal effects is equivalent in the two placental classes (i.e., $\sigma_{e_1}^2 + \sigma_{ee_1} = \sigma_{e_2}^2 + \sigma_{ee_2}$). It is emphasized, however, that this assumption of equality does not necessarily imply that the corresponding components of $\sigma_{E_1}^2$ and $\sigma_{E_2}^2$ are equal (i.e., that $\sigma_{e_1}^2 = \sigma_{e_2}^2$ and $\sigma_{ee_1} = \sigma_{ee_2}$).

If one imposes the restriction that $\sigma_{E_1}^2 = \sigma_{E_2}^2$, the significance of F_W implies

that $\sigma_{e_2}^2 > \sigma_{e_1}^2$ and $\sigma_{ee_1} > \sigma_{ee_2}$, and the nonsignificance of F_A implies that $\sigma_{ge_1} = \sigma_{ge_2}$ or possibly that these latter covariances are zero. In the interpretation of the complex F tests, it should be noted that, assuming $\sigma_{ge_1} = \sigma_{ge_2}$, F'_{A+W} and F'_{A-W} estimate the ratios of linear functions involving $\sigma_{e_2}^2$ and $\sigma_{e_1}^2$, and σ_{ee_1} and σ_{ee_2}. Further, the higher values of F'_{A-W} suggest that the environmental covariances for the two types of MZ twins differ to a greater degree than the environmental variances (i.e., $\sigma_{ee_1}/\sigma_{ee_2} > \sigma_{e_2}^2/\sigma_{e_1}^2$).

In our view, component differences of the type suggested by the observed data on cholesterol level have a reasonable basis. Logically, if there exists a similarity in the prenatal environments of monochorionic pairs which is not present to the same degree for dichorionic pairs, then σ_{ee_1} should exceed σ_{ee_2} and reciprocally, $\sigma_{e_2}^2$ should exceed $\sigma_{e_1}^2$. Also, twin embryos developing in the same uterus may not be in complete developmental synchronization during all stages of the pregnancy. Lack of developmental synchronization might arise under the following circumstances: unequal subdivision of the embryonic cells, the spatial orientation of the fetuses in the uterus, cytoplasmic differences, separation of the fetuses by membranes, or unequal sharing of either the maternal or fetal blood supply. Presumably, a substantial lack of developmental synchronization during critical stages of development could lead to unequal competition for and/or distribution of essential metabolites resulting in significant discordance. It seems plausible that any such developmental asynchrony would tend to be minimized in monochorionic twins by the presence of a single placenta and circulatory anastomosis, but not in dichorionic twins because of their physical and/or circulatory separation which allows each twin to develop at more nearly an independent rate.

The heterogeneity in the within-pair variation of monochorionic and dichorionic twins or of the MZ group as a whole suggests that there may be two populations of MZ twins with regard to variation in cholesterol level. This conclusion was also reached by Jensen et al. [17] from a study of cholesterol variation in adult twins. While they were unable to make a subdivision as to chorion type, it is interesting to note the correspondence between the frequency of markedly discordant twins they observed ($\approx$20%) and the extended frequency of dichorionic twins in the MZ population as a whole ($\approx$30%). Because of our small sample size, it is not possible to attribute the discordance in cord blood cholesterol to chorion type alone. In fact, because of the discrepancy in the linear ($0.886\,\overline{R}_2 = 21.0$) and quadratic ($W^{1/2} = 31.0$) estimates of the intra-pair standard deviation in the dichorionic group as opposed to the close agreement in the monochorionic class ($0.886\,\overline{R}_1 = 12.4$, $W^{1/2} = 13.0$), it may well be that the dichorionic group is composed of two populations with regard to cord blood cholesterol.

In general, the existence of differences in the environmental variances and covariances of monochorionic and dichorionic MZ twins could have important implications for the interpretation of twin data, both with respect to heterogeneity within MZ twins, and more importantly, as a source of extraneous variation in comparisons of MZ and DZ twins. Differences in these environmental components could bias studies where they have not been taken into account or where the proportion of

monochorionic vs. dichorionic sets in the sample is unknown. For this reason and because the degree of transience of chorionic effects on traits such as cholesterol level is unknown, it would seem prudent to examine the MZ class for heterogeneity before any interpretation is made as to the genetic control of the trait [6]. If heterogeneity is found within the MZ class, the data can provide evidence for the influence of prenatal factors on the trait in MZ twins, and both data sets may then be used to establish the range of genetic effects in comparisons with DZ twins. In principle, dichorionic MZ twins should provide the most appropriate subgroups for comparisons between MZ and DZ twins. However, since the dichorionic subgroups will always include any DZ twins which may have been erroneously classified as MZ, extensive use of dichorionic MZ twins for comparisons with DZ twins should only be used where zygosity has been stringently ascertained.

SUMMARY

Cholesterol levels were measured in the cord blood of 30 pairs of monochorionic and 22 pairs of dichorionic monozygotic (MZ) twins. Cholesterol levels were found to be significantly higher in female twins when data was combined over chorion type. The type of chorionic development had no significant effect on variation among twin pairs. Chorion type did, however, have a significant effect on the within-pair variation. The variation within dichorionic pairs was more than five times that within monochorionic pairs ($P < .01$). This result suggests that the variation in placentation has a significant effect on within-pair variation in serum cholesterol of newborn MZ twins.

ACKNOWLEDGMENTS

The authors are indebted to F. Harmath, M. J. Dobbs, and P. Winter for their assistance in the collection and chemical analysis of the samples. The computer facilities of the Department of Medical Genetics and Indiana University Medical Center were extremely helpful in data analysis.

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