Twins in Cardiovascular Genetic Research

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Abstract—Twin studies have been largely responsible for showing the effects of genetic variance on a quantitative trait. The model is based on the fact that monozygotic twins share all genes in common, whereas dizygotic twins are related as siblings and share “on average” half their genes. Environmental confounders are minimized because twin children are usually exposed to similar environments. Blood pressure was first shown to be heritable in a twin study. However, intermediary phenotypes, such as components of the renin-angiotensin system, sympathetic nervous system, renal function, and the facility in excreting electrolytes, are also heritable. The advent of molecular genetics has made twin studies more useful than ever because of the power of quantitative trait loci analyses. Recruitment of the parents of dizygotic twins greatly facilitates this effort. Gene loci linked to blood pressure, intermediate phenotypes, cardiac dimensions, lipid concentrations, and even components of the ECG have been identified. The use of single-nucleotide polymorphisms then allows for novel association approaches within the cohort. Twin studies will continue to gain in importance and utility, particularly in elucidating normal human genetic diversity. (Hypertension. 2001;37[part 2]:350-356.)

Key Words: genes ■ twins ■ genetics ■ polymorphism

In former times, the twin method was regarded as the “royal road” to quantitative genetic analyses in humans. The method was first given a sound foundation by Siemens,1 who showed that twin subjects are by nature very generous persons and can be recruited from schools, universities, and through public advertisement. He also developed an effective method of zygosity testing to distinguish monozygotic (MZ) from dizygotic (DZ) twins. Finally, he was the first to propose comparing traits in MZ and DZ twins, reasoning that MZ twins share all their genes in common, whereas DZ twins share half their genes, being no more similar genetically than other siblings.1 However, like MZ twins, DZ twins are born at the same time and are likely to be exposed to similar environmental conditions. The twin method is based on the fact that MZ twins arise from division of one zygote. Thus, they must be genetically identical or “clones” of one another. Any phenotypic difference between the two must be caused by environmental influences. Environment in this view encompasses anything that is not genetically determined. The method assumes that DZ twins are influenced by largely similar environmental differences as MZ twins but have only half their genes in common by descent. This fact makes them ideal control subjects. The reasoning does have limitations. DZ twins may occasionally develop with an anastamosis of blood vessels, which may lead to mutual transfusion of stem cells. Such twins are chimeras with two populations of genetically different cells. Similarly, monochorionic twins, who are always MZ, can differ as the result of arteriovenous anastomoses, leading to relative malnutrition of one of the twins. Another limitation is possible differences between twins and nontwin siblings. Such differences could impair the general validity of conclusions drawn from a twin sample.

Twin Analysis

Examples of twin analyses from the Berlin cohort pertinent to cardiovascular disease are shown in Figure 1. For instance, the correlation for blood pressure in MZ twins is greater than for DZ twins (upper panel). The same applies for body mass index (lower panel). With classic methodology as we applied in Indianapolis, a genetic influence is demonstrated by a greater “within-pair” variance within DZ twin pairs than within MZ twin pairs. Twin analyses have made major recent advances. In the Berlin twin studies, parameters of the quantitative genetic models were estimated by path analysis techniques with the MX program by Neale.2 Analogous to a regression analysis, the variability of any given phenotype (P) within a population can be separated into genetic influences (A), environmental influences shared by the twins within a family (C), and random environment (E): $P=aA+cC+eE,$ with $a, c, e$ as the estimated relative influence. For MZ and DZ, the covariance of their phenotype is given by $r_{MZ}=a^2+c^2+e^2$ and $r_{DZ}=0.5a^2+c^2+e^2$.

Path analysis in twin studies can estimate additive components of genetic variability (estimated as $a^2$) as well as two environmental influences, shared ($c^2$), and nonshared environmental influences ($e^2$).3 For the purpose of this discussion, we are ignoring nonadditive genetic effects of dominance or epistatis. These values estimate the relative amount of the
influence of the variable on interindividual differences up to a sum of one. Genetic and environmental effects were estimated by the best-fitting model, as selected by the \( \chi^2 \) value.

An example of path analysis as applied to blood pressure during resting state and cold pressor testing is shown in Figure 2. The path model includes two sets of genes, one influencing both resting and stress values (Aa), the second set of genes influencing only the stress values (Ab), two sets of shared environmental factors (Ca and Cb), and two sets of nonshared environmental factors (Ea and Eb), respectively. In addition to comparing the absolute levels of blood pressure at rest and during cold pressor, the bivariate model was applied to the resting level of blood pressure and to the blood pressure responses (\( \Delta BP \)) to stress as well. Because the correlation between blood pressure at rest and blood pressure during cold pressor stress in our subjects was not significant (\( P > 0.05 \)), we used the absolute differences (blood pressure with cold pressor minus blood pressure at rest) as a \( \Delta BP \) value rather than residualized change scores. This type of model tests the hypothesis that blood pressure at rest and the \( \Delta BP \) value with cold pressor stress share genetic variability.

**Quantitative Trait Loci Linkage**

Twin studies lend themselves to molecular genetic investigations both in terms of association and linkage analyses. The power of the twin model in elucidating complex genetic disease was recently emphasized by Martin et al.\(^4\) For linkage studies, the DZ pairs are studied as sib-pairs with the advantage of perfect age matching and reduced environmental variation affecting the phenotype. The MZ twins are used to estimate allele frequencies for the markers tested. The zygosity is verified with microsatellite markers.\(^5\) We have

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**Figure 1.** Within-pair resemblance in MZ and DZ twin pairs for systolic blood pressure (SysBP) (upper panels) and body mass index (BMI) (lower panels). Correlation \( r \) values for MZ twins are \( \approx 0.8 \); \( r \) values for DZ twins are \( \approx 0.4 \). Similarity for MZ twins is significantly greater than for DZ twins.

**Figure 2.** Bivariate path model that separates phenotypic variance and covariance for 2 given phenotypes (ie, defense and substitution) into additive genetic (Ac) and environmental (Ec) contributions that are common to both phenotypes and factors that are specific to 1 phenotype only, additive genetic (As) and environmental (Es) contributions.
Fig. 3. Affected sib-pair approach is shown in which possible distribution of marker alleles in offspring are given provided parents are ab and cd, respectively; 25% of offspring share no alleles in common, 25% share both alleles in common, and 50% share 1 allele in common. Linkage is indicated if allele sharing for IBD1 or IBD2 are increased. For QTL analysis, phenotypic similarity of sibs (measured by the covariance) should increase with the number of alleles they share.

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Cov IBD0 = 0.5 Var addGen, Cov IBD1 = 0.5 Var qtl + 0.5 Var addGen, Cov IBD2 = Var qtl + 0.5 Var addGen.

For linkage analysis, a model is specified estimating Var qtl, Var addGen, and Var env so that the likelihood of the empirical variance-covariance matrix of the sibs, weighted by the probability of sharing zero, one, or two alleles IBD, is maximized. For each sib-pair and each locus, the proportion of alleles IBD, based on parental genotypes, is calculated with a multipoint approach as implemented in MAPMAKER/SIBS. To test for a QTL effect, the absolute difference in variance–covariance –based analysis, nearly 2-fold compared with the independent variable. The high power of the variance-

The use of the structural equation modeling approach as implemented in the MX package. Shown in Figure 3 is an example of two parents harboring distinct alleles at a given locus; each child randomly inherits two. The children may share zero, one, or two alleles identical by descent (IBD). If the locus under study is a quantitative trait loci (QTL), phenotypic similarity of sibs (measured by the covariance) should increase with the number of alleles they share. Assuming no dominance effects, the total variance of the trait is due to the genetic effect of the QTL (Var qtl), the remaining additive genetic effects (Var addGen), and the environmental influences (Var env): Var = Var qtl + Var addGen + Var env. Accordingly, the covariance of the three types of sibs as determined by their IBD status can be predicted as follows: Cov IBD0 = 0.5 Var addGen, Cov IBD1 = 0.5 Var qtl + 0.5 Var addGen, Cov IBD2 = Var qtl + 0.5 Var addGen.

For linkage analysis, a model is specified estimating Var qtl, Var addGen, and Var env so that the likelihood of the empirical variance-covariance matrix of the sibs, weighted by the probability of sharing zero, one, or two alleles IBD, is maximized. For each sib-pair and each locus, the proportion of alleles IBD, based on parental genotypes, is calculated with a multipoint approach as implemented in MAPMAKER/SIBS. To test for a QTL effect, the absolute difference in model fit for models with and without a QTL effect is calculated as a statistic. Because we usually use a candidate gene approach, we accept a value of 0.01 to test for significant linkage in accordance with the criteria defined by Lander and Kruglyak.

We have found utility in combining linkage and association analyses. With linkage, we addressed the question “where is it.” With association (for example, case-control studies), we can formulate the question “what is it,” in terms of candidate genes residing at the locus of interest. Single-nucleotide polymorphisms (SNP) are useful for this purpose. Furthermore, the availability of family structures (individuals and parents) allows for transmission disequilibrium tests and other haplotype-sharing approaches.

Blood Pressure Regulation

Soon after Siemens’s seminal report, twin studies were applied to test the hypothesis that blood pressure is heritable. Numerous excellent twin studies have been done in the area of blood pressure and hypertension research, and they cannot all be mentioned here. Stocks performed one of the first and best twin studies. He investigated English school children, 93 MZ twin pairs, 101 DZ twin pairs of opposite gender, 85 DZ twin pairs of the same gender, 248 sets of brothers and sisters, and 286 sets of same-gender sibships. The intraclass correlation in blood pressure was 0.81 for MZ twins, 0.44 for same-gender DZ twins, and 0.45 for same-gender sibships. His correlations are similar to those we encountered. Twin studies examining blood pressure have since been performed in numerous populations and ethnic groups, demonstrating the universal applicability of twin data in humans.
genetic component was surprisingly strong. Their findings suggest that corticosteroids have a plausible role in essential hypertension that has a similar heritable component.

In Berlin, we found that systolic and diastolic blood pressure were heritable, as were blood pressure responses to cold pressor testing. The path analysis suggested that the genetic influences on resting blood pressure and blood pressure responses to cold pressor were independent of one another. These findings suggested that different genes or sets of genes contribute to blood pressure regulation at rest and blood pressure responses to cold pressor stress.25 We performed a linkage analysis to examine several candidate gene loci.26 We found that systolic blood pressure was linked to the insulin-like growth factor (IGF)-1, Liddle syndrome receptor gene (β-4 subunits of the epithelial sodium channel), and AT1 loci. Linkage to diastolic blood pressure was found at the autosomal-dominant hypertension with brachydactyly gene locus. Both systolic and diastolic blood pressures were linked to the renin gene locus and the β2-adrenergic receptor (β-2 AR) gene locus. The linkage was most consistent for the IGF-1 gene locus and systolic blood pressure. We also found linkage between the IGF-1 gene locus and posterior cardiac wall thickness, septal thickness, and left ventricular mass index.

We elected to focus our attention on the β-2 AR gene.27 We performed an association analysis and found that 4 functionally relevant SNP in the β-2 AR gene, namely Arg16/Gly, Gln27/Glu, Thr164/Ile, and a variant in the promoter region (−47C/T), were associated with blood pressure and heart size differences. The SNP were variably in linkage disequilibrium with each other. A subsequent conditional analysis suggested that the Arg16/Gly polymorphism exerted the predominant effect. These findings underscore the importance of the β-2 AR gene to blood pressure regulation, heart size, and probably to the development of hypertension. We have also shown that blood pressure regulation by baroreceptor reflex sensitivity is under genetic variance.28 Finally, we found that coping skills, not irrelevant to blood pressure, are genetically determined, linked as a QTL to the β-2 AR gene locus, and associated with various polymorphic variants in the gene.29,30

Other investigators have also studied blood pressure–related issues. For instance, the central (aortic) pressure augmentation index has been suggested as a noninvasive measure of pulsatile load, which is a likely determinant of left ventricular mass. Snieder et al31 showed that most of the variance in augmentation index can be explained by genetic and environmental factors specifically influencing the augmentation index. Only a relatively small part of the total variance in augmentation index could be attributed to genes in common with height, heart rate, and mean arterial pressure. Age explained 19% of the total variation in augmentation index. Thus, augmentation index has a significantly heritable component, which is largely independent of the influence of blood pressure, heart rate, height, and age. The responsible genes can be mapped with QTL linkage studies and their variants examined in terms of association.

Jeanclos et al32 recently investigated the effect of genetic variance on telomere length in a twin study. Telomeres, the ends of chromosomes, serve as biological clocks that pace cellular aging in vitro and in vivo. The investigators measured telomere length in 49 twin pairs from Denmark and determined the relation between blood pressure and telomere length. They found that telomere length, and therefore presumably longevity, shows a strong genetic influence. Furthermore, they showed an inverse relation between telomere length and pulse pressure, which in their sample appeared to have a component independent of aging.

The Heart
The diallelic polymorphism in the ACE gene, characterized by a deletion (D) or insertion (I) allele in the 16th intron of the ACE gene, has been associated with differences in plasma ACE levels as well as risk for myocardial infarction and cardiac hypertrophy.33 We conducted a twin study to test the hypothesis that the ACE I and D alleles are associated with plasma ACE levels and that a relation exists between the two and echocardiographically determined cardiac dimensions.

We found an impressive effect of the D allele on plasma ACE levels. Our data also suggested that the ACE gene locus is primarily responsible for ACE plasma levels. We also found a correlation between ACE levels and posterior wall thickness. The D allele was associated with a larger heart.34

Sudden cardiac death in young persons and sudden infant death syndrome are common tragedies in the population. Half of persons with heart failure die of sudden death. Prolongation of the QTc interval, so-called long QTc syndrome, is a well-recognized genetic disease of sodium and potassium channel genes and their regulators. We used the twin model to test the hypothesis that the long QTc gene loci are linked to QTc interval in normal twins. We reasoned that variations in the long QTc genes might well result in minor effects that only have meaning in the face of illness such as heart failure or when medications are ingested that can prolong the QTc. To our surprise, we found that two long QTc gene loci were indeed linked to the QTc interval in the twins and two others were linked to the QRS axis.35 We are now screening these genes for SNP, which we then plan to test in association studies. Along similar lines, we have also used the twin model to test the heritability of heart rate variability, which predicts arrhythmia. Heart rate variability is also heritable, and we were able to show an effect of ACE I/D alleles on this trait.36

Fats and Thrift
Twin studies have been convincing in demonstrating the familial aspects of ischemic heart disease.37 Furthermore, twin studies were pivotal in showing the effect of genetic variance on cholesterol and its fractions.38 We have also used the twin model to test this hypothesis. We found that total cholesterol, LDL, HDL, cholesterol, and triglycerides are all heritable, and we have identified several QTL in this regard.39 For us, the most impressive utility of the twin model came when we mapped a lipid-lowering gene in a family of familial hypercholesterolemia patients who had members with LDL receptor mutations but with nevertheless normal LDL levels. We obtained an LOD score (logarithm of the difference calculated as a likelihood [odds] ratio) of 5 and then checked the same locus in our twin subjects, as shown in Figure 4. The
result was a surprising LOD of 3 in the twins, a finding that was sufficient to convince the reviewers that our findings may be generally relevant.40 This same QTL in the twins is also a QTL for body mass index.

Thrifty genes are probable causes of obesity and type 2 diabetes. The fact that type 2 diabetes is heritable was also first observed in a twin study.41 Because of our interest in body mass index and thrifty genes, we studied the peroxisome proliferator receptor gamma (PPARγ) and its binding partner, the retinoic acid X receptor (RXR).42 We observed that the PPARγ and RXR gene loci were both QTL for lipid values, HDL, and body mass index in the case of PPARγ and triglycerides in the case of RXR. The RXR and triglyceride connection is important because we found that the gene lies directly at a locus implicated in familial combined hyperlipidemia.43 PPARγ interested us even more because we observed that an SNP in the gene showed very few heterozygous DZ individuals; the DZ twins deviated sharply from Hardy-Weinberg equilibrium. MZ twins and non-DZ twin siblings exhibited the expected heterozygosity. Weinberg44 himself had speculated on a genetic cause for DZ twinning. This observation caused us to raise the hypothesis that the PPARγ locus might contain a gene influencing DZ twinning and indeed, with the help of collaborators supplying us with DNA samples from their DZ twins, we found an LOD score of 6.9, as shown in Figure 5.35 Our hypothesis is that if the twins are homozygous for one variant or the other, neither will have an unfair “thrifty gene” advantage, and the chances of both siblings living to term will be enhanced. These observations may shed some light on PPARγ and its functions.

Conclusions
All areas of cardiovascular disease under genetic variance are amenable to further elucidation through twin research. Twin genetics has played a key role in elucidating a host of quantitative genetic hypotheses throughout the history of genetic investigations. A comprehensive and delightful book has recently been published on the topic.46 However, the darker and tragic aspects accompanying the history of genetics, including twin genetics, warrant comment. Galton himself, who is credited with introducing the twin method, had preconceived ideas regarding nature and nurture that contributed to the notion of eugenics. The thought processes of the times are reflected in some of the journal names referenced here.14,41,44 In Germany, the eugenics movement contributed greatly to systematic injustices, sterilizations, and murders, including the mass killings of the holocaust. Some twin geneticists, who were surely aware of these activities, continued their careers after 1945 unencumbered.47 Readers interested in a scholarly monograph on this subject are referred to the work of Müller-Hill and a commentary in that monograph by Watson.48

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Figure 4. Results of linkage analysis for cholesterol-lowering gene in pedigree with familial hypercholesterolemia but normal LDL levels are shown together with linkage results for LDL in DZ twins (probability value transformed into LOD scores). In twins, peak level of significance was 0.0002, on marker D13S1241.
Figure 5. Results of linkage analysis in DZ twin sib-pairs for gene contributing to DZ twinning. LOD score for markers at PPARγ locus approach 7.0.

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