CME

Twin Studies: A Unique Epidemiological Tool

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ABSTRACT

Twin studies are a special type of epidemiological studies designed to measure the contribution of genetics as opposed to the environment, to a given trait. Despite the facts that the classical twin studies are still being guided by assumptions made back in the 1920s and that the inherent limitation lies in the study design itself, the results suggested by earlier twin studies have often been confirmed by molecular genetic studies later. Use of twin registries and various innovative yet complex software packages such as the (SAS) and their extensions (e.g., SAS PROC GENMOD and SAS PROC PHREG) has increased the potential of this epidemiological tool toward contributing significantly to the field of genetics and other life sciences.

Keywords: Concordance rates, molecular studies, statistical analysis system (SAS), twin registries, twin studies

Introduction

The debate of nature versus nurture is known since antiquity. The close resemblance of twins has been the subject of many works of fiction as well. Means of distinguishing between the effects of tendencies received due to genes at birth and those imposed by the different environments they were exposed to during their lives after birth have always been the subject of interest to researchers. The objection to statistical evidence in proof of the inheritance of peculiar traits has always been blamed upon similar environmental conditions playing as a confounder.[1] Twin studies provide a strong basis for exploring the importance of any potential risk factors on a trait or condition by controlling the genetic variations. It has been one of the favorite research tools of behavioral geneticists and psychologists since long, mainly utilized to estimate the heritability of traits and to quantify the effect of a person's shared environment (family) and unique environment (the individual events that shape a life) on a trait.[2]

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Evolution of Twin Studies

The similarity between twins has been a source of curiosity since time immemorial. The idea of using twins to study the heritability of traits can be traced back to the British researcher Sir Francis Galton. His pioneering work *The History of Twins* in 1875 inspired much debate by suggesting that England's "chief men of genius" were the product more of good breeding (nature) than of good rearing (nurture). Based on the similarities he found between twins from 80 questionnaires, Galton proudly announced his conclusion to the world that nature soundly beats nurture, though his sample was too small and consisted of all upper-class individuals, without any control group. After nearly five decades, in the 1920s researchers "perfected' Galton's methods by comparing identical and fraternal twins and inferring heritability from the differences between the two.[3]

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The first reported classical twin study was a study performed by Walter Jablonski in 1922, investigating the contribution of heredity to refraction in human eyes. Jablonski examined the eyes of 52 twin pairs and by comparing the size of within-pair differences between identical and nonidentical twins was able to infer the heritability of a trait.[4]

Even later, in 1990, Thomas J. Bouchard, Jr. and his colleagues (including esteemed twin researcher Nancy L. Segal) at the University of Minnesota conducted one of the most famous research studies on genetic influence in humans. They studied identical twins separated since birth and raised by different families (adoption studies), and so assumed that similarities, if found any, must be those that are heavily influenced by a person's genetic heritage. The study was invoked by the sensational news reports of two identical twins reunited after a lifetime apart. James Lewis and James Springer were separated 4 weeks after birth and each infant was taken in by a different adoptive family. When they were reunited at the age of 39, an extraordinary collection of coincidences emerged. Both of the "Jim twins" had married and divorced women named Linda. Both had second marriages with women named Betty. Both had police training and worked part-time with law enforcement agencies. Both had childhood pets named Toy. They had identical drinking and smoking patterns, and both chewed their fingernails to the nub. Their firstborn sons were named James Alan Lewis and James Allan Springer.[5] Bouchard and Segal reported that about 70% of the variance in intelligence quotient (IQ) found in their particular sample of identical twins was found to be associated with genetic variation. Furthermore, identical twins reared apart were eerily similar to identical twins reared together in various measures of personality, personal mannerisms, expressive social behavior, and occupational and leisure-time interests. However, they did not find outstanding similarities between identical twins on measures such as standardized personality tests. Still, Bouchard's findings can be interpreted as strong support for genetic influences on personality. Bouchard's data set was unique and probably a one-time event in history because modern adoption agencies no longer break up sets of identical twins.[6,7]

The modern-day classical twin study design relies on studying twins raised in the same family environments, which provides control not only for genetic background but also for shared environment in early life. As monozygotic (identical) twins develop from a single egg fertilized by a single sperm, which splits after the egg starts to develop, they are expected to share all of their genes, whereas dizygotic (fraternal) twins share only about 50% of them, which is the same as nontwin siblings.[8] Thus, if any excess similarity is seen between the identical twins when a researcher compares the similarity between sets of identical twins to the similarity between sets of fraternal twins for a trait or condition, then most probably the reason behind this similarity is due to genes rather than environment.

Some assumptions are also made in twin studies; one of them is the assumption of random mating, which assumes that people are as likely to choose partners who are different from themselves as they are to choose partners who are similar for a particular trait. If, instead, people tend to choose mates like themselves, then fraternal twins could share a greater percentage of their genes than expected. In the case of nonrandom mating, fraternal twins would have more genetically influenced traits in common than expected because the genes they receive from their mothers and fathers would be similar to each other. Similarly, the assumption of equal environments is also made, which assumes that fraternal and identical twins raised in the same homes experience similar environments. It is assumed that genes and the environment typically make only separate and distinct contributions to a trait. In general, it is also assumed that only one type of genetic mechanism—usually additive—operates for a particular trait. However, traits can be inherited through different genetic mechanisms. Additive genetic mechanisms mix together the effects of each allele. For example, if genes for curly hair were additive, a curly-haired father and a straight-haired mother might have a child who has wavy hair.[8]

There can be variations in the classical model, which may sometimes provide an added advantage, for example if twins are followed up over longer duration of time in longitudinal manner to assess the development of adult-onset traits and conditions. This slight deviation will allow for a more complete and accurate assessment of environmental factors over time. Similarly, on combining with molecular genetics, information about the presence or absence of specific genetic variants to determine the impact on the trait of interest can be explored. The advances in molecular genetics have substantiated hypotheses generated by the traditional twin research design by pinpointing the effects of a particular gene. Depending on the objectives of the study, one may need only monozygotic or dizygotic twins, or a combination of the two.[8]

Twin Registries: Unique Database

A twin registry is a database of information about both identical twins and fraternal twins, which is often maintained on a country-wide level or by an

academic institution, such as a university or other research institution. There are various twin registries all around the world, including in Sweden, Denmark, Norway, Finland, Australia, Sri Lanka, and the United Kingdom.[9,10]

Registration of some twin registries are mandatory by law, for example Norway, where all births of twins since 1967 have been registered by the Norwegian government.[9,10] However, enlisting with the Australian[11] and Sri Lankan[12] registries is voluntary. The twin registry in some countries have also made extensive outreach efforts, for example examining hospital birth records and then making multiple follow-up efforts such as in-person visits to find the twins and have them agree to be registered.

The Danish Twin Registry is the oldest national twin register in the world, initiated in 1954, and contains information about more than 88,000 twin pairs born in Denmark since 1870, in addition to triplets and quadruplets.[13]

The Danish Twin Registry is used as a source for studies on genetic influence on normal variation in clinical parameters associated with clinical studies of specific diseases, the metabolic syndrome and cardiovascular diseases, and aging and age-related health problems. In all cohorts the ascertainment has been population-based and independent of the traits studied, although different procedures of ascertainment have been employed.[13,14]

The Swedish Twin Registry (STR), managed by the Karolinska Institute, is the largest population-based twin registry in the world (containing approximately 1,70,176 twins in 85,088 pairs born 1886-2000). There are 1,37,414 twins still alive and living in Sweden.[15,16]

It is a unique resource for clinical, epidemiological, and genetic studies. Information has been mainly collected for demographic, medical, and lifestyle characteristics, with special attention to general health, cardiovascular and respiratory disease, legal drug use, and dietary and psychosocial conditions. It is currently in the final phase of a complete telephone interview screening of all twins born in 1958 or earlier regardless of gender composition or vital status of the pair. This effort is known as the Screening Across the Lifespan Twin study (SALT).[15,16]

The famous Minnesota Twin Registry is a registry of all twins born in Minnesota from 1936 to 1955 and from 1961 to 1964; it was started in 1983. The Minnesota Center for Twin and Family Research (MCTFR) presently oversees two longitudinal studies: The Minnesota Twin Family Study (MTFS) and the Sibling Interaction and Behavior Study (SIBS). Both studies include over 9800 individuals comprising twins, siblings, and parents. The MTFS began in 1989, when it enrolled 1,400 pairs of identical and same-sex fraternal twins and their families from the upper Midwest. Twins were identified through public birth records and invited to participate with their parents in a full-day intake assessment. SIBS is a study of adoptive as well as biological sibling and their parents.[17] The primary purpose of SIBS are to understand how siblings interact and influence one another, how family environment has an impact on the psychological health of adolescents, and how adoptive families are similar to and different from nonadoptive families. It is one of the largest studies of adolescents and their families ever conducted.[17]

The Sri Lankan Twin registry (SLTR), established in 1996, is the first ever and only existing population-based Twin Registry in a low- and middle-income country (LMIC).[15] It is presently confined to Colombo district, the most populous among the 25 administrative districts of Sri Lanka. It is comprised of a volunteer cohort of 14,120 twins (7,060 pairs) and 119 sets of triplets, and a population-based cohort of 19,040 (9,520 pairs) twins and 89 sets of triplets. Several studies have been conducted using this registry, including the Colombo Twin and Singleton Study (CoTaSS 1; 4,387 twins, 2,311 singletons), which have explored the prevalence and heritability of a range of psychiatric disorders as well as genetic/environmental interplay. SLTR is a classic showcase of successful North-South partnership in building a progressive research infrastructure in a LMIC.[12,18]

Indian Scenario

Though there are many small-scale twin studies published in various journals related to metabolic syndromes,[19-21] cardiovascular diseases,[22] respiratory diseases, [23] cerebrovascular diseases,[24] epilepsy,[25,26] dermatology,[27,28] ophthalmology,[29,30] psychology[31] chromosomal disorders,[32] and dentistry,[33-36] among others, there exists no twin registry in India to documenting the details of twins borne. In addition, there is no provision of any law for mandatory twin registration. There are many practical problems associated with registering twins borne, one of the important concerns being the large number of home deliveries. In a country where recording the birth weight of every newborn is not yet possible, mandatory twin registration may prove a distant dream for the already overburdened health-work force. Still, outreach activities can be planned to register the twins. Apex medical institutes and tertiary care centers can take the initiative to maintain and analyze data regarding twins in their areas to find out various genetic as well as environmental confounders in various diseases.

Methods Used In Twin Research

The large pool of data related to twins gathered can be analyzed in various ways with the help of new, innovative as well as complex statistical softwares. Twin studies intend to measure the heritability of a trait, which can be determined by concordance rates.

Concordance rate (CR) for a disease or trait among identical and fraternal twin pairs is actually a statistical measure of probability: If one twin has a specific trait or condition, what is the probability that the other twin has (or will develop) that same trait or disease? Historically, CRs are computed separately for monozygotic (MZ) and dizygotic (DZ) pairs. When MZ concordances are greater than DZ concordances, genetic influences are indicated.[37]

Quantitative genetic analyses and heritability estimation, including comparisons of concordances or intraclass correlations and structural equation modelling, can also be used to investigate the relative importance of genetic and environmental influences on a particular trait or condition. Linear structural equations and fit models over all types of twins can be used to describe the causes of variation in a phenotype. Structural equation modelling of data can provide further refinement in the results. The total variance in the trait can be partitioned into genetic variance, common environmental variance including shared (familial) environmental variance, and unique environmental variance. In order to estimate the parameters of interest, the equation for the twins is written and the parameters studied. Heritability, the relative importance of genetic influences for variation in a trait, is defined as genetic variance divided by the total phenotypic variance.

Tetrachoric correlations

It is calculated for two normally distributed phenotypic variables that are both expressed as a dichotomy (disease or no disease) and reflect the similarity of twin pairs. Thus, differences in correlations between various groups provide information about the presence of genetic effects.

Multivariate analyses of twin data can additionally offer estimates of the extent to which allelic variants and environment may influence different traits and conditions.[37]

The co-twin control analyses method is applied in situations where one wants to investigate the importance of an expected risk factor after controlling for genetic and shared environmental effects. It should be noted that the co-twin control method may entail control of factors in the biological pathway between exposure and disease, which may cause an underestimation of the exposure studied.

Co-twin control analyses: Disease-discordant twins

In studies of disease-discordant twins, two control groups usually are used: External controls and internal or co-twin controls. The analysis classically is conducted in three steps.

Step 1: Association between exposure and outcome (comparison with external controls). The first step, which is essentially a classic case-control study, is to compare twins diagnosed as cases with external controls (other twins not related to the index probands), and to evaluate the risk for disease given an exposure. This approach facilitates comparisons with results from ordinary case-control studies on singletons.

Step 2: Controlling for confounding from unmeasured early environment (healthy co-twin as control). In the second step, the healthy co-twin (in both MZ and DZ twin pairs) can be used as a control for the diseased twin. Because twins share the same intrauterine environment and typically are reared together, the co-twin control method provides a very effective tool to minimize confounding by differences in an (unmeasured) childhood or adolescent environment.

If analyses with external controls show associations between exposure and disease and the relative risk remains similarly high in the within-pair (co-twin) analyses, it speaks in favor of a causal effect of the exposure on the disease. On the other hand, if the relative risk is not increased in the within-pair comparisons (but only in the first-step analyses with external comparisons), this indicates that environmental factors early in life (for example, fetal environment, maternal smoking, or childhood socioeconomic status (SES)) are responsible for the initially observed findings. If the relative risks from steps 1 and 2 differ, a direct test of significance of difference in risks can be performed by applying regression: The exposure on control status (external versus internal control).

Step 3: Controlling for unmeasured genetic background (healthy monozygotic co-twin as control). In the third step, analyses are applied only to disease-discordant MZ pairs. This design is ideal in controlling for potential confounding from genetic factors, as the cases and controls are genetically identical. Thus, one is confident that an observed effect is not confounded by genetic predisposition. If the twin with the exposure in MZ pairs more often has a specific chronic disease, this will provide strong support for the likelihood that the exposure contributes to the causation of the disease. On the other hand, if an association exists in analyses of external controls among disease-discordant DZ pairs but not among MZ pairs, genetic effects have probably confounded the results.

Co-twin control analyses: Exposure-discordant twins

As mentioned above, one can also focus on exposure-discordant pairs that are followed longitudinally for a disease outcome. In this case, *t*-tests or proportional hazard regressions can be utilized for estimating the relative risk between exposed and unexposed individuals, whereas matched analyses should be used in within-pair analyses, similar to the disease-discordant pairs.

Finally, as the twin registries contain longitudinal data on large samples, they can therefore be used for conventional epidemiological analyses disregarding twinship status. Several studies have been performed on the association between exposure and outcomes using the registries as a population-based cohort or as the basis for nested case-control studies. When using twin data for these types of studies, the dependency between the twins in a pair should be taken into account by using generalized linear models or other techniques.[15,37,38]

Statistical methods and analysis

Various complex software packages such as Statistical Analysis System (SAS), Mx (Mx is a software developed by (Michale Neale, Department of Psychiatry and School of Medicine, Virginia Common Wealth University, Richmond, VA-23298-0126, USA. Mx is a matrix algebra interpreter and numerical optimizer for structural equation modeling and other types of statistical modeling of data.)) are used for statistical analysis for the twin studies: For applying logistic regression, SAS PROC GENMOD using generalized estimating equation (GEE) model can be used, while for conditional logistic regression, SAS PROC PHREG can be used.[38,39]

Advantages of twin studies

- Twin studies allow disentanglement of the shared genetic and environmental factors for the trait of interest.
- Researchers can estimate the proportion of variance in a trait attributable to genetic variation versus the proportion that is due to shared environment or unshared environment.
- The use of twins can improve the statistical power of a genetic study by reducing the amount of genetic and/or environmental variability; the extent to which different assumptions matter may depend on which trait is being studied.

Limitations of twin studies

- Results from twin studies cannot be directly generalized to the general population, due to lack of randomization; in addition, they are different with regard to their developmental environment, as two fetuses growing simultaneously.
- Some researchers also suggest that genetic factors may lead to a higher incidence of twin births in some women.
- Though lot of changes happened in the field of genetics over time, twin studies today are also based on the same assumptions that were made back in 1920s. Many of these are deeply flawed.
- Findings from twin studies are often misunderstood, misinterpreted, and blown out of proportion, not just by the media, but even by serious scientists who get their work published.
- Many twin registries depend on the voluntary participation of twins. This leads to volunteer bias or recruitment bias, a special type of selection bias, which may lead to overinclusion of identical and female twins, resulting in overestimation of the heritability of the trait or condition under study.
- The use of twins does not allow the researcher to consider the effects of both shared-environment and gene/environment interaction simultaneously. This can be addressed by including additional siblings in the design.

Conclusion

Scholars have long studied twins to address the "nature and nurture" question; however, opposing "nature" to "nurture" is misleading. Genes combine with the environment to produce complex human traits. The importance of genes suggested by earlier twin studies has often been confirmed by later molecular genetic studies. Therefore, twin studies will continue to inform mankind about the relative importance of genes and the environment on traits in ways that no other type of research ever can. Though they have received much criticism, the advancement of statistical techniques (such as structural equation modelling) and the implementation of additional controls have allayed some of the concerns, if not all. The original twin study design has expanded to include studies of twins' extended families, longitudinal studies, and other variations. Some of these variations may allow researchers to address previous limitations also. Many molecular genetic studies have shown the usefulness of twin studies as an exploratory tool, whether or not the assumptions of equal environments and assortative mating are exactly met.

Therefore, twin studies will continue to be an important tool along with emerging genome and molecular research methods in shedding light on various aspects of human genetics and on how environmental factors and genetics combine to create human traits and behaviors.

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Conflicts of interest

There are no conflicts of interest.

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