Agenda

TWINS: GOOD VALUE FOR RESEARCH

Are twins twice as nice or double trouble when it comes to their value in research studies?

BY JOHN WHITFIELD

Researchers who use twins as subjects of their studies refer to their work as research with twins rather than twin research, emphasising the collaboration between subjects and researchers. As a group, twins are willing to go to considerable trouble to help scientific, medical and social research.

But why recruit twins for research projects? Doesn't the fact that they grow up with a co-twin and (at least with "identical" twins) that their parents push them towards similarity make them unsuitable for objective research? Apart from research on the biological, psychological and social aspects of being a twin, what advantages do experimental designs involving twins offer?

There are, in fact, many ways twin studies can and are being used, including biomedical research in Australia. By understanding the appropriate use of twin designs, researchers can avoid some of the pitfalls of twin-based research and use it to make a useful contribution to their work.

Twin study designs

Twin pairs are of two types, monozygotic (MZ) or identical twins and dizygotic (DZ) or fraternal twins. As the names imply, monozygotic twins arise from the division of a single fertilised egg and have all their genes in common, while dizygotic twins result from the release of two ova within the same menstrual cycle, each fertilised by a different sperm. DZ pairs have, on average, half of their nuclear genes in common.

In general, a pair of twins are MZ if people who know them have trouble telling them apart. Information about the number of placentas at or before birth is not reliable. Definitive diagnosis of zygosity (which may be needed if a bone marrow or organ transplant is contemplated) requires extensive blood grouping or DNA tests.

With rare exceptions [1] and regardless of zygosity, pairs of twins grow up with the same social and economic background, live in the same household and mix with much the same people. This similarity of environment, and total or partial similarity of genes, leads to the classical twin study design, in which the relative contributions of genes, shared environmental factors, and non-shared environmental influences, can be assessed.

Disease risk and measurement

Genetics, especially in the medical sphere, deals mainly with genes which cause all-or-none effects. As knowledge of these genes increases, more qualifications about any statement become necessary. We expect that if a person has the gene for an inborn error of metabolism, for example, he or she will show the signs and symptoms of that disease, usually from an early age. In the case of twins, if they are MZ they will be concordant for the condition (both affected or both unaffected).

However, with many common diseases and genetically-influenced characteristics the relationship is not that simple. A multitude of influences, some genetic, some environmental and some random, can all affect the outcome. There are often also measurement or classification errors to take into account.

Risk of disease may be considered as a continuum, with some threshold above which the risk turns into reality. The researcher can do two things: measure twin concordance for the disease to estimate heritability; or measure risk factors (if they are known) to see how much they are influenced by genes or environment. Ideally you would do both because discrepancies would suggest the existence of previously-unknown risk factors.

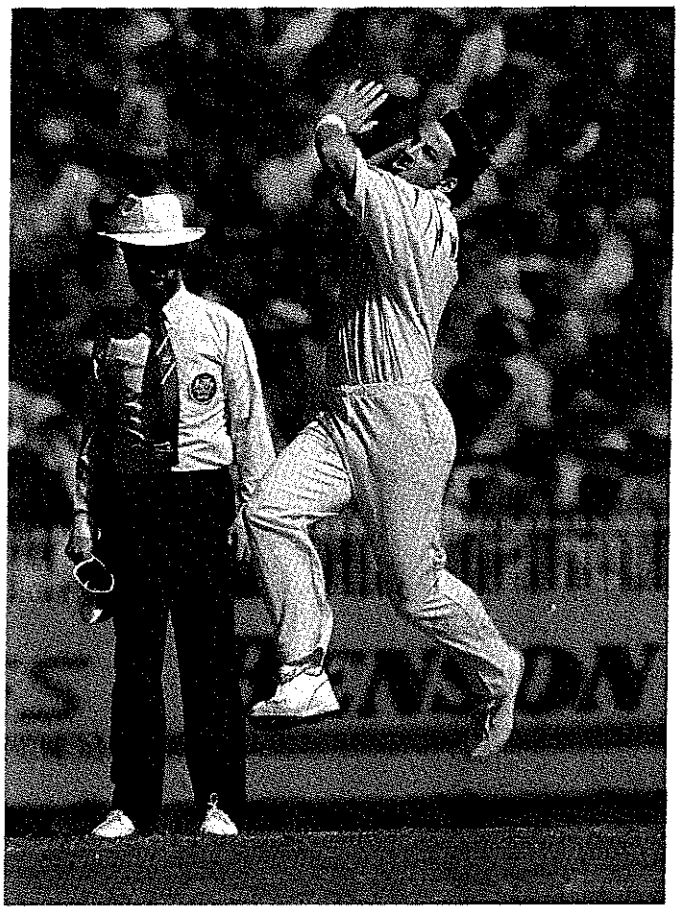
For example, take height as a measurable characteristic. There are some rare inherited conditions which cause a major reduction in height but which do not influence height in the general population. Also, height changes with age so all pairs of twins, regardless of zygosity, will be similar. Furthermore, average height of a population differs between racial groups and can change with time, a factor usually ascribed to improvements in nutrition. So if your aim is expressing the effects of genes as heritability, you need to specify the group studied and any reasons for exclusion from the study.

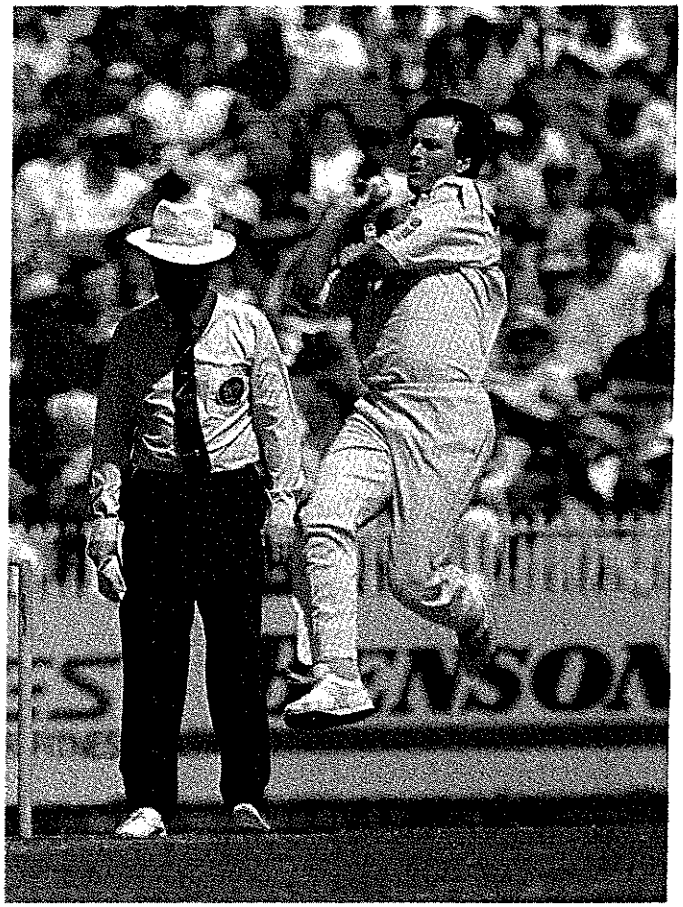
TYPES OF TWIN STUDIES
Classical
Co-twin control
Environmental and genetic correlations between variables
Incorporation of quantitative measures of environmental influences
Genotyping at candidate loci
Confirmation of completeness of explanation of effects
Linkage within sibling pairs

Table 1.

Additive genes and shared and non-shared environments

Twin studies usually break the total variance of the population into three areas: variance due to genetic effects, the effects of environments shared by members of a family, and environmental effects unique to each individual. The shared environmental effects may persist throughout life or they may be short-term and found only





Despite being fraternal twins, the sporting prowess and medium – fast bowling styles of Australian Test cricketers Mark Waugh (left) and Steve Waugh (below) are nearly identical.

when the family is living together. Similarly, the unique environmental effects will be either ephemeral or reproducible across occasions of study.

MZ pairs share both genetic and shared environmental factors. By comparison, DZ pairs are only partially similar for genetic reasons and should be similar for shared environmental reasons to the same extent as MZ pairs. Unrelated pairs of individuals reflect only the overall range of values encountered in the population sampled.

The existence and degree of genetic and shared environmental effects can be tested by comparing the correlation or concordance within MZ pairs to those of DZ pairs. Distinguishing between these two effects, or deciding that both are present, can be difficult unless large numbers of pairs are available [2].

Other types of twin studies

As shown in Table 1, twins are used for a number of types of study with different designs. Co-twin control studies, usually done with MZ pairs, involve creating or exploiting a difference between members of a pair and measuring its effects. Combining the classical twin study design with measurement of genotype or environmental factors suspected to be contributing to variation allows determination of how much of the genetic or environmental variation is due to a specific factor, and whether other factors are also present.

Potential flaws in classical twin studies

Twin studies have been criticised for a number of reasons:

- Some people are uncomfortable with the idea that personality or abilities are inborn, and fear political abuse of information derived from genetic studies in such areas. One of the best-known early twin studies, on intellectual ability, has been claimed to be fraudulent because the author invented data from non-existent subjects.
- The classical method is claimed to be flawed because the environment shared by MZ pairs is more similar than that shared by DZ pairs. Also, twins are not considered typical of the overall population. Each of these points receives serious consideration from twin researchers. Investigation has generally shown that if parents treat MZ children more similarly it is because the children choose more similar environments and/or are more similar in their nature. Whether a genetically-influenced choice of environment should be considered as an environmental or genetic effect is a matter of definition.
- Twins are, in some ways, not typical of the population because they are usually born after a shorter gestation than for singleton pregnancies. This can be checked by each investigator for the measurements of interest and any mean differences from non-twin subjects noted.

A pragmatic approach to these issues

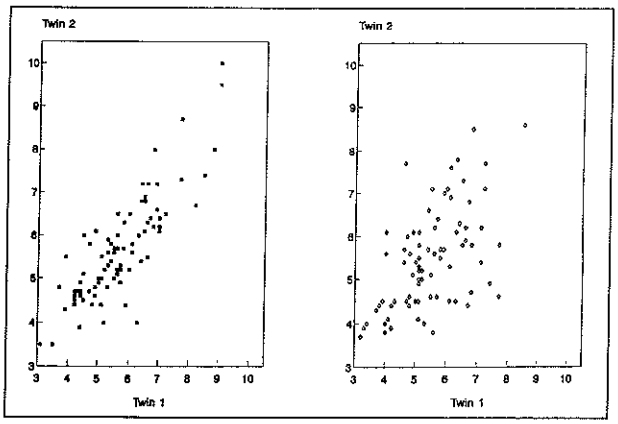


Figure 1. Plasma cholesterol levels show similarities within pairs of twins; but more so for MZ pairs (left) than DZ pairs. Closer analysis of these results revealed that significant shared environment effects occur, at least in these young (18 to 35 year old) twins.

is to integrate results from twin studies with knowledge from other sources. On the whole, results from standard twin studies lead to the same conclusions as more expensive and rarer studies such as: studies using twins reared apart; adoption and half-sibling studies; or multi-generational family studies. Conclusions from twin studies will be upheld or overthrown by identifying (or failing to identify) the genes contributing to MZ pair similarities.

The Australian NHMRC Twin Registry

Because serious twin studies require quite large numbers of subjects, organised twin registries have been developed in many countries. These may be based on birth records or voluntary enrolment by twins. In Australia, a number of independent and small registers of twins were amalgamated into a national grouping in the mid-1980s, which now receives ongoing support as the Australian NHMRC Twin Registry. There are now about 20,000 pairs of twins, of all ages and spread across Australia, in the registry and a large number of studies have been carried out with its aid.

Twins who find out about the registry and are interested in more information can phone the registry for an enrolment form and preliminary questionnaire. Once registered, they receive a newsletter, and as the need arises, invitations to participate in particular projects. Refusal for one study will not prejudice the chance of taking part in another. If they express interest then all the usual ethical considerations of informed consent and freedom to withdraw at any time apply.

From the researcher's point of view, the Twin Registry requires an application form to be completed with information on the project's scientific aims, the number of subjects needed and any special characteristics (eg age, sex, location), and what will be required by the twins. The application is reviewed by two members of the registry's executive and any problems are resolved.

If the investigators have ethical approval from an acceptable institutional ethics committee, an approach letter is sent to suitable twins and those who are interested reply directly to the investigator. There are mechanisms for complaints to be handled by the Registry and if necessary a project may be suspended until any difficulties are resolved.

The twins do not receive any payment for being on the registry nor usually for participating in research projects, so the twins on it are those who are keen to help in research, willing to fill out and return lengthy questionnaires or travel to a hospital or university in their city to take part in an experimental study.

Australian studies

Twin research in Australia is active in a variety of areas (see Table 2). Studies of bone disease and bone density are addressing a problem of great importance as the Australian community ages, and researchers have recently succeeded in identifying and measuring the effects of one genetic (vitamin D receptor polymorphism) and one environmental factor (smoking) which contribute to bone weakness.

Current research areas involving the Twin Registry
Alcohol metabolism and susceptibility to intoxication
Alcohol use and dependence
Allergy and asthma
Bone density, osteoporosis and effects of calcium supplements
Depression
Eating disorders
Heart disease and its risk factors
Hyperactivity
Hypertension
Melanoma and moles

Table 2.

Potential future studies depend mainly on the initiatives of investigators and the number of twins available who have the necessary characteristics. A number of second-order studies can be conducted with a sufficiently large registry;, in these studies twins would need to fulfil some pre-condition such as advanced age, high cholesterol, or hazardous drinking so that they are at risk of the associated diseases.

This would allow studies into areas such as the development of coronary artery disease in subjects with high cholesterol that involve additional genetic or environmental factors. The variation in physical aging in people of similar chronological age could be the subject of a twin study for which only some of the twins on the registry would be suitable.

Another growing area of research for which twin subjects offer advantages is linkage studies. With the increase in genetic markers of known chromosomal location it is now becoming possible to identify loci affecting quantitative traits, even if other loci or non-genetic influences are also important. Large numbers of pairs of siblings are needed for such studies, and DZ twin pairs have the advantages that they are of the same age and from similar environments.

Conclusion

The use of twins as subjects in experimental or epidemiological studies widens the scope of the questions which can be answered. Recruitment of twins is made easier through the NHMRC Twin Registry. Its continuing success and usefulness depends on twins volunteering and on researchers putting forward well thought-out and relevant proposals.

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References
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