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The value of twins in dental research

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Abstract

Due to recent advances in molecular genetics, studies of twins will continue to provide important insights into how genetic and environmental factors contribute to variation in human physical and behavioural traits and disorders. This review emphasizes that biometrical genetic studies of twins are particularly valuable in complementing and directing molecular approaches to facilitate the detection of quantitative trait loci. It also describes several other research models involving twins, apart from the traditional comparison of similarities in monozygotic (identical) and dizygotic (non-identical) pairs, that have the potential to provide new information in the future. Current knowledge about the genetic bases of common dental problems is summarized and future directions in dental research involving twins are outlined.

Key words: Genetics, dental development, dental caries, periodontitis, malocclusion.

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INTRODUCTION

The classical twin research design, involving comparisons of similarities in monozygotic (MZ) or identical pairs and dizygotic (DZ) or fraternal pairs, has enabled researchers to quantify the relative contributions of genetic (nature) and environmental (nurture) factors to variation in many human physical and behavioural features and disorders. Indeed, there have been several recent reviews emphasizing the value of twin studies in clarifying how genetic factors affect common dental problems, such as dental caries, periodontal diseases and malocclusion.[1-4] However, with recent advances in molecular biology and the outcomes of the Human Genome Project, some may feel that twin studies have become less important or have lost their relevance altogether.

This paper highlights research designs involving twins, other than the classical approach, that have the potential to further our understanding of the role of genes in health and disease. It also shows that innovative approaches to the study of twins can complement molecular approaches in unravelling the etiologies of complex diseases and disorders that display multi-factorial, rather than mono-genic, patterns of inheritance. The complex problems of dental caries, periodontal diseases and malocclusion are still the most common reasons for people seeking dental treatment but their genetic bases remain unclear.

Zygosity determination

In many of the early twin studies there were problems associated with inaccurate diagnosis of zygosities. Although comparisons of physical features, for example body build, facial appearance, eye colour and ear form can provide a reasonably accurate means of distinguishing between MZ and DZ twin pairs, mistakes can still be made (Fig 1). Furthermore, asking relatives or twin pairs themselves will not always lead to correct zygosity determination. Following the discovery of blood groups and enzyme polymorphisms, it became possible to determine whether twins were monozygotic or not with relatively high probability. More recently, with advances in DNA technology, a small number of highly polymorphic DNA markers can be used to establish zygosity with very high probability. Rather than obtaining blood samples to extract DNA, it is now possible to isolate DNA from cheek cells obtained using buccal swabs.

The MZ co-twin design

A powerful approach to clarify the roles of genetic and environmental influences on normal features, or susceptibility to diseases, involves studying MZ twin pairs who show different phenotypic expressions for a particular trait or disease under investigation. Alternatively, researchers can manipulate the environment so that each member of a twin pair is exposed to different environmental conditions.[5] Monozygotic twin pairs are matched perfectly for age and sex and, as they share the same genes except in very rare circumstances, any differences between them will normally reflect environmental effects.[6] As Martin *et al.*[6] have pointed out, it is often assumed that these environmental factors are exogenous to the individual, e.g., related to lifestyle, but a variety of endogenous events leading to alterations in cellular development

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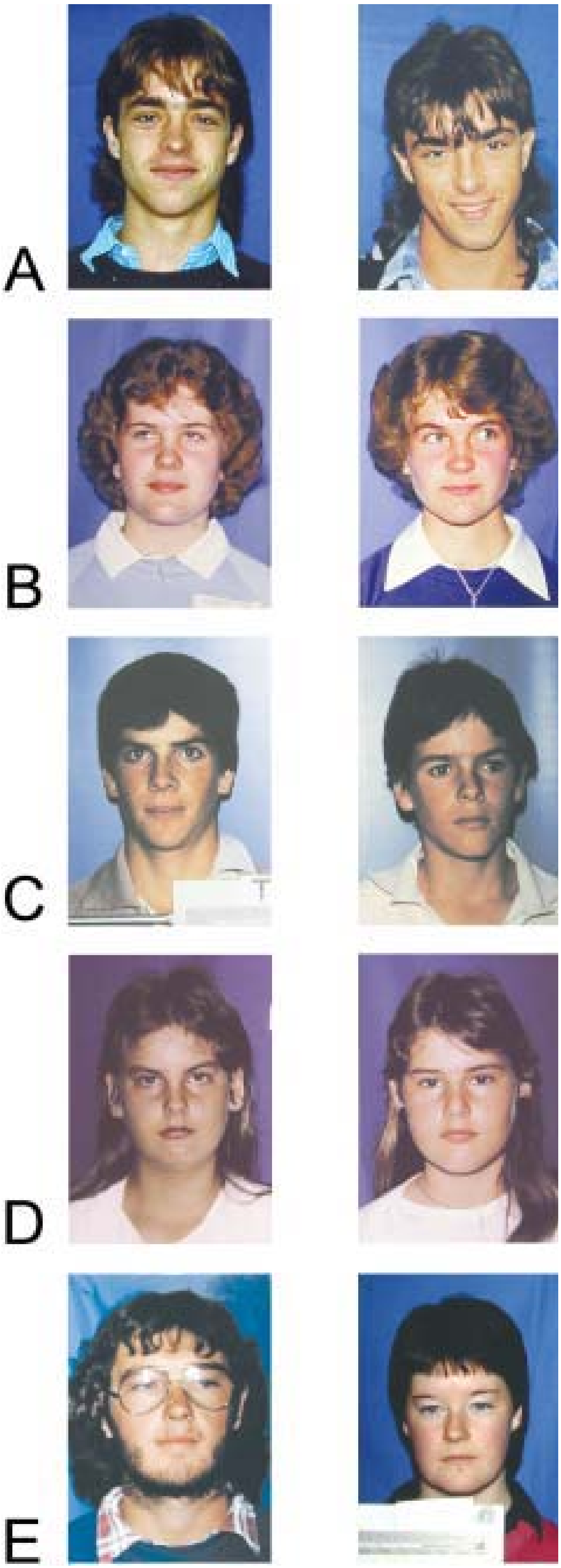

Fig 1. Determining the zygosity of twin pairs on the basis of their facial appearance is not always a simple matter. A and B are MZ twin pairs, C and D are DZ twin pairs, and E are opposite-sexed DZ twin pairs.

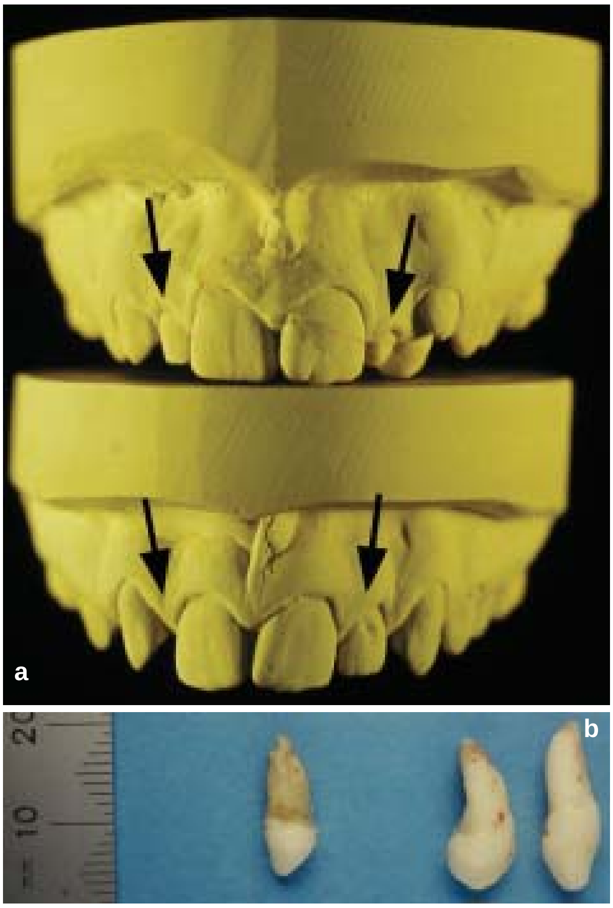

Fig 2(a). Dental models demonstrating asymmetrical expression of upper lateral incisor morphology in a pair of monozygotic (MZ) female twins. The model above shows a diminutive right lateral incisor and a peg-shaped left lateral incisor. The model below shows a missing right lateral incisor and a normal-sized left lateral incisor. (**b**) Supernumerary teeth removed from the anterior maxillary region of both members of a pair of MZ male twins – one supernumerary from one of the twins and two supernumeraries from the other.

and differentiation, e.g., from differences in the timing or degree of methylation of autosomal genes, may also lead to differences in phenotypic expression between MZ twin pairs.

A dental example is that of variation in expression of missing, peg-shaped, diminutive and normally-formed upper lateral incisors within pairs of MZ twins (Fig 2a). These associated features conform to a multi-factorial threshold model linking tooth size with presence and absence of teeth. Several examples have been reported in Australian MZ twin pairs where one member of the pair displays agenesis of one or more upper lateral incisors, whereas the other shows diminutive or peg-shaped teeth.[7] Another example of different phenotypic expression of a dental feature within MZ twin pairs is varying expression in the number of supernumerary teeth (Fig 2b).[8]

Another approach using this model that has been reported in the orthodontic literature, is the provision of different treatments to the two members of an MZ twin pair and observation of the outcomes.[9] Given that considerable insights can be gained by applying this

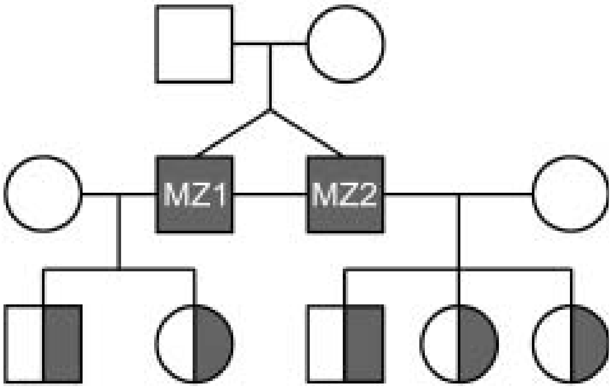

Fig 3. Pedigree showing that the offspring of MZ male pairs (denoted by a closed triangle) with unrelated partners share half their genes on average – they are genetically half-siblings.

model to only one set of twins, the MZ co-twin method is a feasible and potentially valuable approach to apply within a dental practice.

Twins reared apart

The ongoing studies of Tom Bouchard and his colleagues in Minnesota involve examination of MZ twin pairs who were separated at birth and then reared apart in different surroundings.[10] This model overcomes one of the often mentioned limitations of traditional twin studies – that MZ twin pairs may be more similar than DZ pairs for particular features, not because they share more genes, but because they share more of the environmental factors that influence the trait in question. Over 80 pairs of MZ twins who were reared apart from birth are enrolled in Bouchard's study that addresses an array of behavioural and biomedical features. Studies of MZ twin pairs reared apart have confirmed the important role played by genes in both dental morphology and caries experience.[11]

Opposite-sexed DZ twins

If zygosity and sex are considered, there are five main groups of twin pairs that may be studied: MZ female pairs, MZ male pairs, DZ female pairs, DZ male pairs and DZ male-female pairs. Comparison of correlations between same-sex DZ pairs and opposite-sexed (OS) DZ pairs provide an opportunity to investigate whether primary or secondary sexual characteristics are affected by diffusion of hormones in utero. There is indirect evidence that diffusion of hormones may occur in humans via the maternal circulation[12-14] and more direct evidence from rats, pigs, monkeys and cows.[15-16] There is also evidence that testosterone can diffuse across the amniotic membranes separating rat fetuses.[17]

Several studies of twins have revealed evidence for masculinization of human females from OS pairs, but not feminization of males from these pairs. Traits that have been shown to display this effect are verbal ability,[18] mathematical performance and perceptual speed,[19] spatial ability,[20] and sensation-seeking behaviour, including adventure seeking and susceptibility to boredom.[21] Comparisons of dental crown diameters in females from same-sex DZ pairs with females from (OS) DZ pairs has revealed that the latter have larger teeth, on average. However, no consistent difference has been noted for similar comparisons in males.[22] Diffusion of sex hormones from male to female co-twins in utero may account for the increased tooth size in OS females.

The twin half-sib model

This model involves making comparisons between the offspring of MZ pairs and their partners (Fig 3). Potter[23] emphasized the potential value of the twin half-sib approach and indicated that it could be applied not only for partitioning shared genetic and environmental risks within families, but also for determining the involvement of maternal effects or assortative mating. The advantage of this model is that the children of MZ pairs who are born to different mothers are themselves genetically half-siblings.

As Potter[23] pointed out, shared behaviour and habits within families may contribute to similarities between relatives. For example, common dietary patterns and oral hygiene practices within families may account for similarities in dental disease rather than the sharing of genes. Because MZ twin pairs have identical genotypes, their children will share, on average, half their genes whether they have the same twin as a parent or not. Monozygotic pairs, their spouses and their offspring share different levels of genetic relationship, ranging from zero for the spouses of twins in the absence of assortative mating, to unity for the twins themselves. There are also interesting environmental relationships, as the children of identical twins are socially first cousins who normally live in different home environments. They are also likely to be more similar in age than half-siblings in families formed following the death of a spouse or divorce. Despite its advantages, including efficiency in relation to sample size,[24] Potter's[23] encouragement to researchers to use the half-sib model to study common dental diseases has so far gone unheeded. However, Harrap and colleagues have used the model productively in a study of cardiovascular risk factors in Australians.[25]

Linkage studies

As Martin *et al.*[6] note, there have been important advances in the field of human quantitative genetics over the past decade or so. The concepts of biometrical genetics have been addressed by multivariate analyses, such as factor analysis, to enable the genetic basis of co-variation between groups of traits to be explored. The first applications of this approach were made using LISREL, a structural equation modeling package, but Neale[26] has now produced a programme, Mx, that can be used for multivariate genetic modeling and for quantitative trait loci (QTL) linkage analysis.

Quantitative trait loci represent segments of DNA that have an influence on variation of one or more measurable traits. Dizygotic twin pairs, who are matched for age, provide an excellent group from which to screen and ascertain discordant sibling pairs for QTL analysis.[27] With the availability of genome scans becoming more widespread, the likelihood of discovering QTLs relevant to a wide range of traits and disorders is increasing, but only those of large effect can be detected with the sample sizes typically collected to date. Nevertheless, it should be possible to detect QTLs for tooth size in the not-too-distant future. After QTLs have been detected by linkage analysis, the next challenge will be to identify and clone the genes themselves.

Special features of twins

An underlying assumption of twin studies has been that their results can be extrapolated to the singleton population. However, some have questioned whether this is appropriate and have highlighted the special circumstances of the twinning event and the pre-natal environment in which twins develop. Twinning has been associated with a high peri-natal mortality rate[28] and there is also a higher frequency of congenital abnormalities in MZ twins, many of which seem to be related to failure of bilateral structures to fuse during development.[29]

Martin *et al.*[6] acknowledge that certain disorders are more common in twins, particularly in MZ twins, but claim that the potentially harmful effects of twin gestation have been over-emphasized. However, of considerable interest are the results of ultrasound scanning that indicate a large proportion of twins, possibly as high as 80 per cent, fail to develop past 16 weeks post-conception. This has prompted reference to the so-called 'vanishing twin' phenomenon and raises the question of how many single births may actually represent surviving twins.[30] Interestingly, recent data from researchers at Monash University have thrown the whole 'vanishing twin' concept into question.[31]

It is important to realize that MZ pairs can be categorized into different groups depending on their placentation (Fig 4). If the placenta, chorion and amnion are all doubled, it is likely that the zygote has cleaved at some time between one to five days post-conception, prior to implantation. About 20-30 per cent of MZ pairs fall into this category and are termed di-chorionic. If there is a single placenta and chorion but double amnion, it indicates that cleavage is likely to have occurred between six to nine days post-conception, after implantation. Approximately 65 per cent of MZ pairs fall into this category and they are referred to as mono-chorionic.

In about 30 per cent or more of mono-chorionic twin pairs, arterio-venous anastomoses can lead to marked physical discrepancies. For example, it has been suggested that a large birth-weight difference (say >450g) between the two members of an MZ pair can be used as an indicator of the transfusion syndrome and, by inference, late cleavage of the zygote.[32] In a small

Fig 4. MZ twin pairs may be categorized into different groups depending on their placentation.

percentage of twins (around 3 per cent) the placenta, chorion and amnion are all single, indicating that cleavage has probably occurred between nine to 10 days after conception. Further delay in cleavage produces conjoined, or Siamese twins, a rare event that occurs approximately once in every 400 MZ pair births.

Mirror-imaging

The dentitions and faces of twins provide a good opportunity to study the fascinating phenomenon of mirror-imaging, in which one twin mirrors the other for one or more features (Fig 5).

There is some preliminary evidence that mirror-imaging may be related to the timing of the twinning event and therefore the type of placentation, but these findings were based on the use of birth-weight differences between the members of MZ twin pairs to retrospectively infer mono-chorionicity.[33] Unfortunately, few twin studies have been carried out in which placentation data have been obtained, and none that we are aware of have looked at the association between placentation and mirror-imaging.

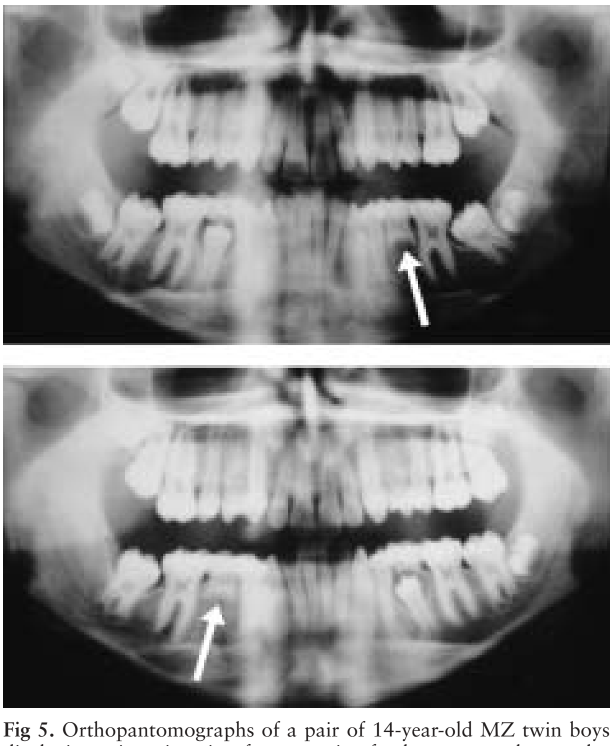

Fig 5. Orthopantomographs of a pair of 14-year-old MZ twin boys displaying mirror-imaging for agenesis of a lower second premolar (arrowed).

Discordance in handedness, where one member of an MZ twin pair is right-handed and the other left-handed, can be considered to be another example of mirror-imaging. It is presumed that these differences in handedness reflect variations in cerebral lateralization between twins. Approximately 30 per cent of MZ twin pairs have been reported to be discordant for handedness, an intriguingly high percentage given that MZ twin pairs share the same genes.[34] However, whether all cases result from the same underlying biological cause remains to be seen.

The frequency of non-righthandedness in twins, including left-handers and mixed-handers is higher in both MZ and DZ twins than in the general population (16-20 per cent compared with 10 per cent respectively).[34] It has been reported that non-righthandedness is also elevated in the parents and siblings of twins.[35] This has prompted the suggestion that there may be some common mechanism within certain families that accounts for both left-handedness and the twinning event.[29] However, further research is needed to confirm whether this is so.

The genetic basis of common dental problems

Studies using different twin designs have indicated that the risk of dental caries has a genetic basis, but these investigations have not been able to specify which of the different etiological factors, diet, saliva, tooth morphology or microbial flora are most important.[4] Analyses of twins reared apart have provided the strongest evidence that genetic factors contribute to caries incidence, as the confounding issues of common environmental effects within twin pairs are overcome. Although twin studies have provided strong evidence of a genetic contribution to dental caries risk, they have not provided any evidence of linkage to, or association with, specific genes.[4] Genetic linkage studies based on well-defined populations are identified by Schuler[4] as the necessary next step in analyzing the relationship between inheritance and dental caries.

A recent investigation of periodontitis and gingivitis in 64 MZ twin pairs and 53 DZ twin pairs reared together confirmed that approximately 50 per cent of the variance in adult periodontitis could be attributed to genetic factors.[36] In contrast, there was no evidence of heritability for gingivitis after behavioural covariates, such as smoking and dental care, were taken into account. Although the precise biological basis for the greater similarity in periodontitis between MZ twin pairs compared with DZ twin pairs remains to be elucidated, approaches in which the twin model has been applied *in vitro* hold considerable promise.[1] For example, by obtaining cells such as fibroblasts from twins and exposing them to various periodontal pathogens and other agents associated with periodontitis, researchers are now in a position to determine the genetic basis of susceptibility to disease at a cellular level.

As Mosey[3] has pointed out, 'the key to the determination of the aetiology of malocclusion, and its treatability, lies in the ability to differentiate the effect of genes and environment on the craniofacial skeleton in a particular individual'. Twin studies have confirmed that genetic influences contribute to variation in occlusal traits to varying degrees, with tooth size and arch shape displaying relatively high heritabilities, whereas heritability estimates for inter-arch variables such as overbite and overjet are considerably lower.[37] However, these findings have not led to major changes in the clinical management of malocclusions. By applying new morphometric methods of analysis to 3D cranio-facial data derived from longitudinal studies of twins, our understanding of how genes influence normal growth processes will improve. Twin studies of bone density that are providing new insights into the timing of growth and genetic contribution to skeletal development of Australian children may also find application in dentistry.[38] By linking these approaches with the techniques of molecular genetics, exciting new possibilities for prevention and management of dental malocclusion should arise.

The Australian Twin Registry

The Australian Twin Registry, which is supported by the National Health and Medical Research Council of Australia, was formed in the 1980s after the amalgamation of a number of smaller registers. Over 30 000 pairs of twins and higher-order multiples are now enrolled in the Registry. Monozygotic and DZ twins of all ages are participating in a wide range of approved research projects addressing many important issues, for example, alcohol metabolism, allergies and asthma, bone density and osteoporosis, epilepsy, depression, eating disorders, heart disease, hyperactivity, hypertension, melanoma, prostate disease, breast cancer, diabetes and dental development. The web address for the Australian Twin Registry is http://www.twins.org.au and the email address is dph-twins@unimelb.edu.au.

The future

With the advances that have occurred in both human quantitative genetics and molecular biology over the past decade, we are now in a position to build a more complete understanding of how our genes and the environment contribute to a range of human diseases and disorders that display multi-factorial etiologies. Far from being out-moded, well-designed twin studies have a central role to play in this new era. They can throw new light on how genes influence developmental mechanisms and they can also provide efficient ways of helping in the search for QTLs.

Our challenge as dental researchers and clinicians will be to translate the knowledge we will soon have about the genetic basis of disorders affecting the oral hard and soft tissues into improved preventive and treatment strategies for the community at large.

Apart from their value in clarifying how genetic factors influence oral diseases and disorders, studies of the teeth and faces of twins and higher- order multiples can also provide insights into basic biological processes, such as the determination of body symmetry and the role of hormones on intra-uterine development. The dentition in particular provides a very useful model system to investigate developmental mechanisms, given that teeth begin to develop soon after conception and then form in an orderly sequence over an extended period of time. Once formed, teeth are not re-modelled, so they can be used to make retrospective assessments of how developmental disturbances affect morphogenetic processes both pre- and post-natally.

Multi-disciplinary studies of twins, with input from dentists, molecular geneticists and twin researchers, hold great promise for the future, not only in clarifying how genetic factors contribute to oral diseases and disorders, but also in unravelling the mysteries of how our body symmetry is determined. Observations by dentists and auxiliaries of twin pairs presenting at private or public dental clinics can also provide valuable insights into how genes and the environment interact during development.

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