Our studies of the teeth and faces of Australian twins commenced at the School of Dentistry, The University of Adelaide in the early 1980s. There are now over 900 pairs of twins enrolled in our continuing investigations, together with 1200 relatives. There are 3 main cohorts of participants. The first cohort comprises around 300 pairs of teenage twins for whom various records have been collected, including dental casts, facial photographs, finger and palm prints and information on laterality, including handedness. The second cohort comprises around 300 pairs of twins who have been examined at 3 stages of dental development from approximately 4 years of age to about 14 years: at primary, mixed, and permanent dentition (excluding 3rd molars) stages. The most recent study of tooth emergence and oral health, for which we are currently recruiting twins, will provide a third cohort of around 500 twin pairs aged from around birth to 3 to 4 years of age. Our broad aim in these studies has been to improve our understanding of how genetic and environmental factors contribute to variation in dental and facial features, and to oral health. We have also used our data to investigate aspects of the determination of laterality, particularly the fascinating phenomenon of mirror imaging. We plan to maximize the use of the longitudinal data and DNA we have collected, and continue to collect, by performing genome-wide scans for putative genetic linkage peaks for a range of dental features, and then to test for association between a series of likely candidate genes and our phenotypes.

Brief History
Research relating to human growth and dental anthropology in the University of Adelaide's Dental School dates back to the early part of the 20th century when Draper Campbell published his landmark thesis entitled ‘Dentition and palate of the Australian Aboriginal’ (Campbell, 1925). Subsequently, Murray Barrett and Tasman Brown carried out a longitudinal growth study of Aboriginal Australians living at Yuendumu in the Northern Territory of Australia. Over 1700 sets of dental casts of approximately 450 subjects were obtained and these records have enabled detailed analyses of dental development to be carried out, including tooth emergence and the formation of the dental arches (Brown & Townsend, 2001). The compilation of genealogical records for this population also enabled genetic analyses to be performed, with particular reference to tooth size (Townsend, 1980; Townsend & Brown, 1978). These studies confirmed a relatively strong genetic influence on variation of dental crown size but also showed that environmental factors played a role.

In the early 1980s, a study commenced of the teeth and faces of teenage twins living in Adelaide, South Australia. The main aim was to quantify the relative contributions of genetic and environmental factors to variation in dental and facial features. Many papers have been published based on the data generated from investigations involving this first cohort of twins. For example, it has been shown that there is a strong genetic contribution to human tooth size variation, with smaller common and unique environmental components of variance (Dempsey & Townsend, 2001).

The study was expanded in the 1990s to include collaboration with Professor Louise Brearley Messer at The University of Melbourne. A second cohort of young twins with primary teeth was recruited to allow genetic analyses focusing on dental and facial growth and development. This longitudinal study aimed to collect records at three key times of dental
development: at the stage when all primary teeth were present (around 3–5 years), when children had mixed dentitions (around 8–10 years), and when all the permanent teeth were present except third molars (around 12–14 years). Collection of records is now almost complete and several analyses have been completed or are underway, including some with collaborators from Japan and the United States (Corruccini et al., 2005; Richards et al., 1997).

Most recently, a third cohort of twins is being recruited for a study of tooth emergence and oral health. This study involves an Australia-wide recruitment approach with key collaborations between investigators in Adelaide (led by Professor Townsend), Queensland (Associate Professor Seow) and Western Australia (Professor Gotjamanos). The project is focused on clarifying the extent to which genetic factors contribute to variation in the timing and sequence of emergence of primary teeth. We are also determining the time at which mutans streptococci (MS), key organisms in the development of dental caries, first colonize the oral cavity of young twins. We are interested in determining whether initial colonization is linked to the emergence of primary teeth. An additional aim of this study is to clarify the pattern of transmission of MS within family members.

Recruitment Procedures
We have worked closely with the Australian Twin Registry and the Australian Multiple Birth Association to recruit twins for our studies. We have also actively recruited twins pairs for the third cohort from newspaper birth announcements, hospitals, and prenatal exercise classes. Retention rates throughout the studies have been high with less than 10% attrition. We do not make any cash payments to participants but we do provide dental products, gifts and vouchers, as well as offering advice on oral health and reporting on any relevant findings from oral examinations. The following e-mail addresses are given as a first point of contact for anyone interested in our studies: sandra.pinkerton@adelaide.edu.au or michelle.bockmann@adelaide.edu.au.

Table 1 summarizes the sample sizes for the three cohorts of twins. At present, there are 913 pairs of twins enrolled in our ongoing studies. In addition, we have collected information for around 1200 family members. Nearly all of the participants are of European ancestry.

Zygosity Determination
Zygosities of those twins examined in the 1980s were confirmed by comparisons of a number of genetic markers in the blood (ABO, Rh, Fy, Jk, MNS) together with several serum enzyme polymorphisms and protein polymorphisms. Zygosities of twins in cohort 2 have been confirmed by analysis of up to six highly variable genetic loci (FES, vWA31, F13A1, THO1, D21S11, FGA) on six different chromosomes, using DNA obtained from buccal cells. Determination of zygosity for twins in cohort 3 is being done using nine highly variable genetic loci on nine different chromosomes.

Ethical Issues
Our investigations have the approval of the Committee on the Ethics of Human Experimentation, The University of Adelaide (Approval Nos. H-07-84A, and H-78-2003) and all participants are informed volunteers.

Data Available for the Three Cohorts of Twins
The following types of records and information have been collected in our studies.

Cohort 1
- oral examinations of all participants to record teeth present and to detect any evidence of dental caries or other problems
- alginate impressions of the upper and lower dental arches that were cast in good quality dental stone

Table 1
Twin Pairs Enrolled in Studies of Teeth and Faces of Australian Twins

<table>
<thead>
<tr>
<th></th>
<th>MZ♂</th>
<th>MZ♀</th>
<th>DZ♂</th>
<th>DZ♀</th>
<th>D♂♀</th>
<th>Total†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st cohort</td>
<td>73</td>
<td>86</td>
<td>43</td>
<td>51</td>
<td>56</td>
<td>309</td>
</tr>
<tr>
<td>2nd cohort</td>
<td>84</td>
<td>79</td>
<td>50</td>
<td>54</td>
<td>60</td>
<td>327</td>
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<tr>
<td>3rd cohort</td>
<td>45</td>
<td>41</td>
<td>33</td>
<td>22</td>
<td>67</td>
<td>208</td>
</tr>
<tr>
<td>Zygosity analysis in progress</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>69</td>
</tr>
<tr>
<td>Total</td>
<td>202</td>
<td>206</td>
<td>126</td>
<td>127</td>
<td>183</td>
<td>913</td>
</tr>
</tbody>
</table>

Family Members Enrolled

<table>
<thead>
<tr>
<th></th>
<th>Mothers</th>
<th>Fathers</th>
<th>Brothers</th>
<th>Sisters</th>
<th>Other</th>
<th>Total</th>
</tr>
</thead>
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<tr>
<td>1st cohort</td>
<td>65</td>
<td>52</td>
<td>19</td>
<td>35</td>
<td>7</td>
<td>178</td>
</tr>
<tr>
<td>2nd cohort</td>
<td>51</td>
<td>27</td>
<td>104</td>
<td>87</td>
<td>4</td>
<td>273</td>
</tr>
<tr>
<td>3rd cohort</td>
<td>279</td>
<td>257</td>
<td>124</td>
<td>89</td>
<td>—</td>
<td>749</td>
</tr>
<tr>
<td>Total</td>
<td>395</td>
<td>336</td>
<td>247</td>
<td>211</td>
<td>11</td>
<td>1200</td>
</tr>
</tbody>
</table>

Note: † plus 8 sets of triplets.
• intraoral and extraoral photographs, together with standardized photographs of faces
• stereophotogrammetric records of facial features
• palm and fingerprints, as well as information about laterality including hand, foot and eye dominance
• detailed medical histories of the twins
• height and weight measurements
• blood samples for DNA analysis and zygosity determination.

Cohort 2
Similar records to those obtained for cohort 1 but with the addition of:
• buccal swabs for DNA extraction, zygosity determination and future linkage studies
• standardized facial profile photographs.

Cohort 3
• primary tooth emergence data
• microbiological swabs of saliva/dental plaque from twins and family members
• buccal swabs for DNA extraction, zygosity determination and future linkage studies
• detailed questionnaires on medical histories of mothers and twins, oral health histories, feeding habits, and so on
• clinical examinations of selected individuals.

Data Collection and Methods of Analysis
Data for analysis have been acquired by several methods, including directly measuring or scanning dental casts, and by digitising landmarks on standardized photographs of individual teeth, dental arches and facial photographs. Many different variables have been analyzed including dental crown size, intercuspal distances, dental arch size and shape, occlusal variables such as overbite and overjet, and various facial dimensions. We have reported the results of replicability studies that have shown that errors of the methods are generally small and unlikely to bias results (Eguchi et al., 2004; Townsend et al., 2003).

The equipment for stereophotogrammetric studies of facial morphology in our first cohort of twins consisted of matching left and right Hasselblad motor-driven cameras mounted on a rigid frame with an attached acrylic template fixed at a distance of 1000 mm from the film plane of the cameras. We obtained five stereo views of the face for each subject — frontal, left and right profiles, and left and right half-profiles (Brown et al., 1987). For the second cohort of twins, we have obtained standardized profile photographs of the faces of twins using a custom-built orthogonal photographic system in the School of Dental Science at The University of Melbourne (Tangchaitrong et al., 2000).

We have used the generalized structural equation modeling program, MX, developed by Neale et al. (2002) to carry out genetic analyses of dental data in our twin sample (Dempsey et al., 1995). Heritability estimates ($h^2$), calculated as the ratio of additive genetic variation to total phenotypic variation, have been calculated for several different dental and facial phenotypes (Hughes et al., 2000, 2001a, 2001b).

The dental and facial traits examined so far have been chosen to represent increasing levels of complexity within the dentition. We have considered factors influencing variation in individual teeth, including intercuspal distances and crown features such as Carabelli trait (Townsend et al., 2003; Townsend & Martin, 1992). We have also explored how genetic and environmental factors influence variation in the positioning of teeth within and between the dental arches, as well as in selected facial features (Townsend et al., 2006).

Apart from using the traditional twin approach, involving comparisons of monozygotic (MZ) twin pairs and dizygotic (DZ) twin pairs, we have also used the MZ co-twin design to investigate genetic and environmental influences on dental traits where one twin shows a feature and the other shows different expression or does not display the trait at all (Townsend et al., 2005). We have also looked at tooth size in opposite-sexed DZ twin pairs to determine whether there is any evidence for a possible hormonal influence on dental development in utero (Dempsey et al., 1999a). Although we only have information on chorion type for a small proportion of our twin samples, we have been able to conduct some preliminary investigations of the relationship between chorion type, birthweight and tooth size (Race et al., 2006).

Teeth, faces and fingerprints are particularly suitable for studies of symmetry and asymmetry, and we have been particularly interested in studying the fas-
• Models incorporating additive genetic variance (A) and unique environment variance (E) provide the best fits for most of the dental traits that we have studied to date, although models including only environmental variance, either unique environment (E) alone, or a combination of common and unique environment (C and E) provided the best fits for some molar intercuspal distances. Furthermore, models incorporating common and unique environmental effects (C and E), in addition to an additive genetic effect (A), have provided the best fits to explain variation observed in molar crown diameters (Table 2). Heritability estimates for those variables displaying significant additive genetic variance differ considerably—from 28% for incisal overjet to 92% for maxillary arch length. Intercuspal distances and occlusal traits have shown relatively high phenotypic variation but low to moderate heritabilities. In contrast, crown diameters and arch dimensions have shown relatively low phenotypic variation but moderate to high heritabilities (Townsend et al., 2006).

• Studies of opposite-sexed (OS) DZ twin pairs have indicated that tooth size of females from OS DZ twins tends to be larger than those of females from same-sexed DZ pairs or from MZ females. This finding provides some support for the concept that diffusion of sex hormones from male to female cotwins in utero may account for the increased tooth size (Dempsey et al., 1999a).

• Studies of facial dimensions using Fourier analyses have provided evidence of significant genetic contribution to facial convexity, facial depth and facial height. Variability in nose and lip morphology appears to be under stronger environmental influence (Vanco et al., 1995).

• Studies based on facial photographs of twins have shown the more symmetric twin of a pair is rated consistently as more attractive, and the magnitude of the difference between twins in perceived attractiveness is directly related to the magnitude of the difference in symmetry (Mealey et al., 1998).

• No evidence of a significant role for genetic factors in the determination of handedness has been found in our twin sample, and birth factors do not appear to have a significant impact either (Dempsey et al., 1999b).

• Several dental features, including missing or supernumerary teeth, have shown to be expressed differently in MZ twin pairs. For example, we have noted evidence of at least one missing upper lateral incisor or second premolar in 24 of 278 pairs of MZ twins, with 21 of these 24 pairs showing discordant expression. We have postulated that minor variations in epigenetic events during odontogenesis may account for these distinct differences (Townsend et al., 2005).

• We have recently completed a preliminary genetic analysis of the timing of emergence of the primary lower central incisors, teeth which usually emerge first in an infant’s mouth. The sample comprised 26 pairs of MZ twins and 23 pairs of DZ twins, all of European ancestry, aged from 3 to 12 months. No significant differences were noted in mean emergence times between boys and girls, or between right and left sides (pooled mean = 8.6 months, SD = 2.3 months). The model that best explained observed variation included an additive genetic effect (A) and environmental effects (C and E).
genetic component (A) and a unique environmental component (E). Estimates of their contributions to phenotypic variation were 96% and 4% respectively. These preliminary findings suggest that genetic factors are of considerable importance in determining variation in human primary tooth emergence.

- Testing of bacteria from twins in our third cohort has shown that MS retains significant viability for 9 days after sampling but some problems with yeast overgrowth have been noted with older samples. Nearly all of the adult samples have shown presence of MS. A small number of twins have displayed colonization prior to emergence of any teeth. An MS strain library is being established with future plans to use polymerase chain reaction (PCR) to identify MS species and establish familial transmission patterns from a subset of families.

Discussion and Future Directions
As far as we are aware, our collection of dental and facial records of Australian twins and their families is one of the largest in the world. We have extended our initial morphological investigations of the dentition to longitudinal studies of dental development and oral health. Results of our investigations are expected to provide an important foundation to underpin the move to a more biologically based, preventive approach to dental practice. Our studies are also of relevance to more basic biological issues, including the determination of body symmetry, as well as in the fields of physical anthropology and forensic odontology.

Our analyses have shown that there is a strong genetic basis to observed variation in many human dental phenotypes and we now plan to use a combination of genetic association and genetic linkage approaches to identify the key genes involved in dental development. To the best of our knowledge, this will be the first study aimed specifically at identifying genes associated with a range of clinically relevant phenotypes in the human dentition. Identifying key genes for dental development in humans would provide clinicians with a sounder scientific basis for monitoring and counselling individuals predisposed to developmental problems (e.g., missing teeth, malocclusions), especially where there is a familial history.

In the last decade, many genes have been identified that regulate epithelial–mesenchymal interactions in developing teeth, and the application of both genetic modelling methods and molecular approaches is heralding an exciting new era in craniofacial research. Our focus is to maximise use of the longitudinal data and DNA already collected by performing a genome-wide scan for putative genetic linkage peaks for a range of dental features, and subsequently, inform by linkage, to test for association between a series of likely candidate genes and our phenotypes.

Acknowledgments
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References


