Variation in the human dentition: some past advances and future opportunities

Grant Townsend[1,2,4], Lassi Alvesalo[2,3,4] and Alan Brook[2,4]

[1] School of Dentistry, The University of Adelaide, Adelaide, South Australia, 5005

[2] School of Dental Sciences, The University of Liverpool, Liverpool, UK, L69 3GN

[3] Institute of Dentistry, University of Oulu, Oulu, Finland, FIN-90220

[4] International Collaborating Centre in Oro-facial Genetics and Development, University of Liverpool, Liverpool, UK, L69 3GN

KEY WORDS: teeth, genes, phenotypes, twins, sex chromosomes

For correspondence:

Professor Grant Townsend

School of Dentistry

The University of Adelaide

South Australia, 5005

Email: grant.townsend@adelaide.edu.au

Phone: 61 8 8303 5968

Fax: 61 8 8303 3444

INTRODUCTION

The purpose of this article is to share with the dental research community milestones and creative insights that make dental research what it is today. We have been involved in dental research for some 40 years and have been fortunate enough to see astounding advances in the field of craniofacial biology.

Each of us has been influenced by different mentors during our careers and we have pursued different but complementary research initiatives. Now, an exciting new international collaboration is underway using the unique materials available in Adelaide and Oulu and innovative 2D and 3D imaging techniques for data acquisition in Liverpool. Together we are developing new study designs, recording new dental phenotypes and applying novel aetiological modelling methods, utilising association and linkage approaches. In this paper we review some key influences and achievements that have led to our present collaboration, set out plans for the future and offer to collaborate with others.

THE AUSTRALIAN CONNECTION

The School of Dentistry at the University of Adelaide has a long and distinguished history of research in the fields of dental anthropology and human growth, dating back to T Draper Campbell whose monograph, "Dentition and Palate of the Australian Aboriginal" remains a classic text (Campbell, 1925). Regular visits to the Aboriginal settlement of Yuendumu, located north-west of Alice Springs, Australia, took place from the 1950s to the 1970s. Murray Barrett and Tasman Brown assumed leadership roles in a longitudinal growth study of the Aboriginal children from around 6 to 18 years of age, supported by a grant from the National Institute of Dental

Research, United States Public Health Service. The growth records collected during field trips to Yuendumu continue to provide a unique resource for dental scientists and anthropologists interested in studying facial and dental development in a group of Aboriginal people with only limited exposure to European customs and living conditions. There are over 1700 sets of dental casts for over 450 individuals in the Adelaide collection, together with genealogical and other growth records, including stature and weight measurements.

In 1970 Townsend, a third-year dental student, accompanied Barrett and Brown on one of their annual field trips to Yuendumu, leading to an honours degree and a PhD thesis on the genetic basis of tooth size under Brown's supervision. The realization that collections of high quality growth records derived from well-designed longitudinal growth studies, like the one at Yuendumu, could provide such an invaluable resource over so many years, led Townsend and colleagues to commence studies of dentofacial morphology and development in Australian twins in the 1980s (Townsend et al., 2006a).

There are now over 1,000 pairs of twins enrolled in the Australian twin studies, with three different cohorts represented. In analysing data derived from the twins, advantage has been taken of the major developments in multivariate genetic modelling methods (Neale and Cardon, 1992). The ability to isolate DNA from buccal cells, as well as blood, has also facilitated zygosity determination. Genetic studies have shown that additive genetic contributions to phenotypic variation differ for different dental features, being strongest for tooth emergence, dental crown size and Carabelli trait but weaker for intercuspal distances and occlusal traits such as anterior overbite and overjet (Hughes et al., 2007). In addition to applying the traditional twin model to dental data, the Adelaide group has also analysed data from opposite-sexed

dizygotic twins (Dempsey et al., 1999). The monozygotic co-twin design has been used to highlight the apparent role of epigenetic influences on dental development, including the expression of missing and extra teeth.

THE FINNISH CONNECTION

Alvesalo studied dentistry at the University of Turku, Finland and following a summer period as a dental assistant in 1963 on Hailuoto, an island close to Oulu in Northern Finland, he suggested to his mentor, Kalevi Koski, that Hailuoto would be an excellent location to collect dental records for genetic purposes. In 1966, he examined 669 Hailuoto islanders out of a population of 1265. Extensive records were obtained and genealogies were constructed by geneticist Petter Portin. Alvesalo's 1971 thesis "The influence of sex chromosome genes on tooth size in man" was based on a correlative study of cousins and siblings. It was concluded that the Y chromosome apparently affected tooth crown size, but that its effect differed from that of the X chromosome. Alvesalo also proposed that observed sexual dimorphism in tooth crown size was connected with the influence of the Y chromosome (Alvesalo, 1971).

These findings were the impetus for Alvesalo to commence the large-scale Kvantti project in Finland involving individuals with sex chromosome anomalies and also their close relatives. To date, 314 patients and 371 relatives have been examined, with records including karyotypes, dental casts, panoramic and lateral head radiographs, bitewing radiographs, special enamel radiographs, facial photographs, intraoral photographs, measures of oral health and anthropometric measurements. It has been found that the sex chromosomes have modifying effects not only on tooth crown size, but also tooth shape, structure and root size, as well as influencing

craniofacial form and body size and shape (Alvesalo et al., 1975; Alvesalo, 1997; Lahdesmaki and Alvesalo, 2004). The Kvantti results also showed that the Y chromosome promotes both dentin and enamel growth, whereas the effect of the X chromosome seems to be limited to enamel (Alvesalo and Tammisalo, 1981; Alvesalo, 1985; Alvesalo et al., 1991). These differential effects of the X and Y chromosomes on cell function and proliferation, especially that of the Y chromosome on cell proliferation, may be related to the sexual dimorphism observed in tooth number, average crown and root size, crown morphology and, assuming genetic pleiotropy, other somatic features such as statural growth and sex ratio at birth (Alvesalo, 1997). A number of questions arise regarding the manner and extent of this influence of the Y chromosome on growth (Alvesalo, 1997).

In 1972 Alvesalo was invited to participate in the research project "Genetic-odontometric study of pre- and neonatal growth" led by Richard H Osborne, which formed part of a larger project conducted by the National Institute of Neurological Disorder and Stroke, U.S.A. Dental casts and intra-oral photographs were collected from over 2000 children, including about 200 pairs of twins. In 1988, after his retirement, Osborn shipped all the dental records and medical information to Alvesalo. Analyses performed subsequently at Oulu indicated that mothers' smoking leads to a reduction in tooth size of their children (Heikkinen et al., 1992) and that preterm children's teeth are also smaller in size but the eruption of their permanent teeth is advanced (Harila-Kaera et al., 2003).

THE ENGLISH CONNECTION

Brook was an undergraduate at Guy's Hospital where the teaching and research in dental anatomy of Jeff Osborn was a particular inspiration. After qualifying, he

performed clinical research on the normal and anomalous development of the dentition leading to a thesis on the prevalence, clinical features, associations and aetiology of dental anomalies of number, size and shape by means of a large epidemiological and family study at Eastman Dental Hospital, London. He constructed an aetiological model for anomalies of number and size which could be tested statistically (Brook, 1984). This multifactorial model is based on a continuous scale, related to tooth number and size, with thresholds. Position of an individual on the scale depends on a combination of the additive effect of numerous genetic and environmental factors. Within this background a single gene of major effect, a chromosomal anomaly or a major environmental insult may greatly influence an individual's position on the scale, taking them beyond a threshold with the development of an anomaly.

This model stimulated a series of hypotheses that have been subsequently tested, first with hand measurements and subsequently using 2D image analysis. The studies confirmed the presence of sex differences in tooth size, of smaller teeth and reduced form in hypodontia, but larger teeth and altered form when supernumeraries are present. Studies of a large sample of Romano-British skulls from Pounbury, Dorset, England have shown the same underlying patterns, yet with smaller tooth sizes and higher prevalences of hypodontia compared to the modern British population, possibly associated with major environmental stressors such as poor nutrition, chronic lead ingestion and recurrent infections (Brook and Johns, 1995). As an example of a single gene of major effect in the multifactorial aetiological model, mutations of PAX 9 associated with hypodontia have been described (Das et al., 2003). Subsequent

studies in one of these families have confirmed the effect of this mutation on tooth size as well as tooth number.

In the aetiology of enamel defects, an epidemiological and family study of enamel defects in London schoolchildren identified both genetic and environmental factors. Studies of X-linked amelogenesis imperfecta identified different mutations in AMELX and recently a large X-chromosomal deletion associated with microphthalmia, linear skin defects and amelogenesis imperfecta has been described (Hobson et al., 2008). Major environmental factors including recurrent infections, excess lead ingestion and poor nutrition may have been involved in the much higher frequency of hypoplastic enamel defects found in Romano-Britons compared to a modern British population. These findings are compatible with the multifactorial aetiology model for enamel defects proposed by Brook (1999).

A further ongoing area of development has been new methodologies to improve clinical phenotyping, thereby enabling differences between individuals to be quantified more accurately to enhance aetiological studies. There have been contributions to new diagnostic clinical indices for anomalies (Brook et al., 2001) and the development and validation of 2D image analysis and 3D laser scanning techniques.

Another ongoing theme involves multidisciplinary collaboration, with examples including input from statisticians to describe the complexity of tooth shape (Robinson et al., 2002), and to explain the distribution of hypodontia around the arch, including the relationships of tooth position and type.

A NEW COLLABORATIVE INITIATIVE

The 6th International Symposium on Dental Morphology held in Reykavik, Iceland in 1983 provided an opportunity for Townsend and Alvesalo to meet in person for the first time. A long-term collaboration developed that was re-energized and re-focussed when Alan Brook, organiser of the 12th International Symposium on Dental Morphology in Sheffield in 2001, proposed the establishment of a three-way international research collaboration. Brook's subsequent appointment at the University of Liverpool, the establishment of the International Collaborating Centre in Oro-facial Genetics and Development with Brook as Director, and the support of the University in appointing Alvesalo and Townsend to limited-term professorships and as Associate Directors of the International Collaborating Centre, is now enabling these plans to come to fruition.

Collections of dental casts and other growth records such as those housed in Adelaide and Oulu are unique and are unlikely to ever be replicated for other human groups. Enormous improvements in computing power and development of 2D and 3D imaging techniques are opening up new opportunities for revisiting many research questions that were posed in the past but have remained unresolved due to limitations in the quality and quantity of data that could be derived and analysed.

THE FUTURE

Genome-wide association studies are now possible, enabling identification of genes associated with common human diseases and disorders of complex aetiology. It is now feasible to apply genetic linkage and association analyses to dental phenotypes,

with data from twins and their relatives being particularly valuable (Boomsma et al., 2002).

Rather than being limited to simple 2D measurements of dental crown and arch dimensions, for example maximum mesiodistal and buccolingual crown diameters, we can now capture the occlusal surfaces of teeth in 3D digital form, generate surface contours and compare images mathematically. Scanning micro-tomography permits examination of other aspects of the dental tissues, including enamel and dentine. These new technologies open up new opportunities to address key basic biological and clinically important research questions.

Developments in molecular biology are providing a much clearer picture of the processes involved in odontogenesis, including how crown shape is determined, as well as why certain teeth develop in certain regions of the oral cavity. Signals from the primary enamel knot are instructive for the formation of secondary enamel knots which represent the sites of future cusp tips, and the arrangement of cusps is determined by a balance of inductive and repressive signalling molecules that are produced by the knots (Miletich and Sharpe, 2003). In fact, development of cusps seems to involve repeated activation of the same set of developmental genes, with the precise location of the cusps being determined by a cascade of epigenetic events rather than being under strict genetic control (Jernvall and Thesleff, 2000).

While the original morphogenetic field theory was a useful rule-of-thumb to describe patterns of variation observed within the dentition, recent molecular studies have led to the description of an odontogenic homeobox code to explain dental development in terms of differential gene expression. Mitsiadis and Smith (2006) proposed that the field, clone and homeobox code models could all be incorporated into a single model to explain dental patterning, so that these three models should be

viewed as complementary rather than contradictory. This unifying view can be extended into the clinical setting using findings on dental patterning in individuals with missing and extra teeth.

After an international workshop and symposium on normal and abnormal development of the dentition in November 2007, with a group of researchers joining the International Collaborating Centre network, we agreed to share resources and expertise to enable some key research questions to be explored.

These include:

-What are the most appropriate phenotypes, relating to tooth number, size, form and structure, that can help us understand further the complex aetiologies of dental anomalies in humans? For example, what are the interactions between different genotypes and environmental agents such as fluoride and tetracycline in determining the phenotype?

-How do key genes on both the autosomes and sex chromosomes that have been identified as being involved in normal human dental development relate to and interact with those associated already with craniofacial and dental anomalies in humans and experimental animals?

-What is the specific mechanism by which the Y chromosome promotes growth? Does the increase in mitotic potential due to the Y chromosome promote the penetrance of normal genes or inhibit that of defective genes involved in dental development, e.g. leading to sexual dimorphism in the number and size of the teeth?

-Is the Y chromosome involved in the mineralization process? Are enamel and dentin growth regulated by the same gene within the Y chromosome?

The amino acid sequences of the X and Y amelogenin genes (AMELX, AMELY) seem to differ to some extent and the transcriptional products of the X and Y

chromosomes are both quantitatively and qualitatively different. The Y chromosome locus encodes a functional protein even though its level of expression is only 10% of that of the locus on the X chromosome.

We recognise that there are other centres of excellence also involved in this fascinating and rapidly developing area of research. Our hope is that this review will encourage further productive collaborations in addressing the many important research questions in craniofacial and dental development.

ACKNOWLEDGMENTS

We wish to express our sincere appreciation to all of the participants who have agreed to take part in our studies to date, and to all of our colleagues for their support and contributions over the years. We would also like to thank the various funding bodies who have supported our research including the National Health and Medical Research Council of Australia, the Australian Dental Research Foundation, The Academy of Finland, The University of Turku Foundation, Emil Aaltonen Foundation, The Wellcome Trust, The Royal Society of Medicine and Biotechnology and Biological Sciences Research Council.

REFERENCES

Alvesalo L (1971). The influence of sex-chromosome genes on tooth size in man. Proc Finn Dent Soc 67:3-54.

Alvesalo L (1985). Dental growth in 47,XYY males and in conditions with other sex-chromosome anomalies. In: The Y chromosome, Part B: clinical aspects of Y chromosome abnormalities. Sandberg AA, editor. New York: Liss, pp. 277-300.

Alvesalo L (1997). Sex chromosomes and human growth. A dental approach. Hum Genet 101:1-5.

Alvesalo L, Tammisalo E (1981). Enamel thickness in 45,X females' permanent teeth. Am J Hum Genet 33:464-469.

Alvesalo L, Osborne R, Kari M (1975). The 47,XYY male, Y chromosome, and tooth size. Am J Hum Genet 27:53-61.

Alvesalo L, Tammisalo E, Townsend G (1991). Upper central incisor and canine tooth crown size in 47,XXY males. J Dent Res 70:1057-1060.

Boomsma D, Busjahn A, Peltonen L (2002). Classical twin studies and beyond. Nat Genet 3:872-882.

Brook AH (1984). A unifying aetiological explanation for anomalies of human tooth number and size. Archs Oral Biol 29:373-378.

Brook AH (1999). A unifying model for the aetiology of enamel defects. In: Dental Morphology 1998, Proceedings of the 11th International Symposium on Dental Morphology. Mayhall J, Heikkinen T, editors. Oulu: Oulu University Press, pp.128-132.

Brook AH, Johns CC (1995). Dental anomalies of number and size in a Romano-British population. In: Proceedings of the 10th International Symposium on Dental Morphology. Radlanski R, Renz H, editors. Berlin: "M" Marketing Services, pp.177-180.

Brook AH, Elcock C, Hallonsten A, Poulson S, Andreasen J, Koch G, Yeung CA, Dosanjh T (2001). The development of a new index to measure enamel defects. Dental Morphology 2001. Brook A, editor. Sheffield: Sheffield Academic Press, pp. 59-65.

Campbell TD (1925). Dentition and palate of the Australian Aboriginal. The Hassell Press, Adelaide.

Das P, Hai M, Elcock C, Leal SM, Brown DT, Brook AH and Patel PI (2008). Novel missence mutations and a 288-bp exonic insertion in PAX9 families with hypodontia. Am J Med Genet 118:35-42.

Dempsey PJ, Townsend GC, Richards LC (1999) Increased tooth size in females with twin brothers: evidence for hormonal diffusion between human twins in utero. Am J Hum Biol 11:577-586.

Harila-Kaera V, Heikkinen T, Alvesalo L (2003). The eruption of permanent incisors and first molars in prematurely born children. Europ J Orthod 25:293-299.

Heikkinen T, Alvesalo L, Osborne R, Pirttiniemi P (1992). Maternal smoking and tooth formation in the foetus. I. Tooth crown size in the deciduous dentition. Early Hum Dev 30:49-59.

Hobson GM, Gibson CW, Aragon M, Yuan Z, Davis-Williams A, Kirkham J and Brook AH (2008). A large X chromosomal deletion is associated with microphthalmia with linear skin defects and amelogenesis imperfect. Am J Med Genet (submitted).

Hughes TE, Bockmann MR, Seow K, Gotjamanos T, Gully N, Richards L, Townsend G (2007). Strong genetic control of emergence of human primary incisors. J Dent Res 86: 1160-1165.

Jernvall J, Thesleff I (2000). Reiterative signalling and patterning during mammalian tooth morphogenesis. Mech Devel 92: 19-29.

Lahdesmaki R, Alvesalo L (2004). Root lengths in 47,XYY males' permanent teeth. J Dent Res 83:771-775.

Lench NJ and Brook AH (1997) DNA diagnosis of X-linked amelogenesis imperfecta (AIHI). J Oral Pathol Med 26:135-7.

Miletich I, Sharpe PT (2003). Normal and abnormal dental development. Hum Molec Genet 12, Review Issue 1:R69-R73.

Mitsiadis T, Smith MM (2006). How do genes make teeth to order through development? J Exp Zool (Mol Dev Evol) 306B:177-182.

Neale MC, Cardon LR. (1992). Methodology for genetic studies of twins and families. Kluwer Academic Publishers: Dordrecht, The Netherlands.

Robinson DL, Blackwell PG, Stillman EC, Brook AH (2002). Impact of landmark reliability: on the planar Procrustes analysis of tooth shape. Archs Oral Biol 47:545-554.

Townsend GC, Richards LC, Messer L, Hughes T, Pinkerton S, Seow K, Gotjamanos T, Gully N, Bockmann M (2006a). Genetic and environmental influences on dentofacial structures and oral health: studies of Australian twins and their families. Twin Res Hum Genet 9:727-732.