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Townsend, Grant Clement; Richards, Lindsay Clem; Hughes, Toby Estcourt; Pinkerton, Sandra Kay; Schwerdt, Wendy  
[Epigenetic influences may explain dental differences in monozygotic twin pairs](#) Australian Dental Journal, 2005; 50(2):95-100

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# Epigenetic influences may explain dental differences in monozygotic twin pairs

GC Townsend,\* L Richards,\* T Hughes,\* S Pinkerton,\* W Schwerdt\*

## Abstract

**Background:** Comparisons between monozygotic (MZ) co-twins have tended to focus on the similarities between their dentitions rather than differences. The aim of this study was to determine the prevalence of discordant expression for simple hypodontia and supernumerary teeth in MZ twin pairs and to explain how phenotypic differences might occur despite their similar genotypes.

**Methods:** Records of 278 pairs of MZ twins, including dental casts and radiographs, were examined and the prevalences of discordant expression for missing upper lateral incisors (ULI) or second premolars (PM2), and of mesiodentes, were determined. Zygosity was confirmed by comparisons of blood markers and DNA.

**Results:** There was evidence of at least one missing ULI or PM2 in 24 of the 278 MZ pairs (8.6 per cent), with 21 of these 24 pairs (87.5 per cent) showing discordant expression. Nine of the 278 MZ pairs (3.2 per cent) displayed evidence of mesiodentes, with eight of these nine pairs (88.9 per cent) being discordant.

**Conclusion:** Our findings show that differences in the expression of missing or extra teeth occur often between MZ co-twins whose genetic make-up predisposes them to simple hypodontia or mesiodentes. We postulate that minor variations in epigenetic events during odontogenesis may account for these distinct differences.

**Key words:** Hypodontia, supernumerary teeth, twins, dental development.

**Abbreviations and acronyms:** DZ = dizygotic; MZ = monozygotic; PM2 = second premolars; ULI = upper lateral incisors.

(Accepted for publication 2 July 2004.)

## INTRODUCTION

Classical twin studies involve comparing features of interest in large numbers of monozygotic (MZ) twin pairs with those in dizygotic (DZ) twin pairs. Assuming

that environmental influences are the same in both groups, greater similarity between MZ twin pairs, who share the same genes, compared with DZ twin pairs, who only share half their genes on average, indicates that genetic factors are contributing to observed variation. Applications of this model to dental features have confirmed that there is a strong genetic contribution to variation in human dental morphology,<sup>1</sup> and so researchers and clinicians have often tended to focus on the dental similarities between MZ twin pairs rather than their differences. However, we have reported previously that MZ twin pairs can show quite different expressions of normal, small, peg-shaped and missing maxillary incisors, despite having the same genetic make-up.<sup>2</sup> We have also reported on a pair of MZ twin boys who displayed different numbers of supernumerary teeth, one twin having a single supernumerary and the other having two.<sup>3</sup>

In this paper, we focus on variations in expression of hypodontia for selected permanent teeth, as well as variations in the number of supernumerary teeth within MZ twin pairs. The modes of inheritance for missing and extra teeth in humans are still not clearly established. Pedigree studies of families showing missing teeth have indicated an autosomal dominant mode of inheritance,<sup>4</sup> although autosomal recessive<sup>5</sup> and X-linked modes of inheritance<sup>6</sup> have also been suggested. A polygenic model with both genetic and environmental influences seems to provide the best explanation for observed variation. Brook<sup>7</sup> proposed a multifactorial model linking tooth size and tooth number, with superimposed thresholds, to account for the different patterns of expression of both missing and extra teeth observed in males and females. He found that the relatives of affected individuals were more likely to display missing or extra teeth, supporting an underlying genetic predisposition. He also noted that males tended to have larger teeth and a higher frequency of supernumeraries compared with females who had smaller teeth on average and a higher frequency of dental agenesis. This supported the concept of a link between tooth size and tooth number.

To date, molecular studies in humans have focussed on locating the genes associated with missing teeth

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rather than extra teeth. Over 100 genes are associated with dental development, so any of them could be a candidate for hypodontia.<sup>8</sup> Finnish researchers looked for evidence of linkage between hypodontia and several candidate genes thought to have important roles in odontogenesis and were able to exclude EGF, EGFR and FGF-3, and probably FGF-4, as possible sites for gene mutation in the families they studied.<sup>9</sup> They also excluded the homeobox genes, MSX1 and MSX2 as causative loci for hypodontia<sup>10</sup> but others have suggested that there may be a connection.<sup>11</sup> Recently, genome-wide searches have found an association between the PAX9 gene and oligodontia.<sup>12</sup> Although the precise location of the genes involved in simple hypodontia remains unknown, ongoing genome-wide searches are likely to enable loci to be assigned in the near future.

Even when the genes associated with missing and extra teeth are identified, we will still need to clarify the relationship between an individual's genetic make-up and their phenotype. Epigenetics, a term that, in its broad sense, refers to alterations in gene expression without changes in nucleotide sequencing, is critical in this regard but our understanding of these events remains far from complete. Although molecular geneticists often focus nowadays on specific examples of epigenetic events, for example methylation and acetylation of DNA, we use the term in its broad sense in this paper.

Comparisons of MZ twin pairs who share the same genes but show differences in phenotypic expression provide one means of clarifying how epigenetic influences can affect phenotypic expression. Differences in tooth number between co-twins, in particular, represent distinct and readily observable discordant features. Therefore, our aim in this study was to determine the prevalence of discordant expression for selected missing teeth (upper lateral incisors and second premolars) and the prevalence of discordant numbers of supernumerary teeth (mesiodentes) in a large sample of MZ twin pairs. We then attempt to explain the reasons for the phenotypic differences between their dentitions despite their similar genotypes.

## MATERIALS AND METHODS

Records of 278 pairs of twins, including panoramic radiographs and dental models, were examined and the prevalence of congenitally missing upper lateral incisors (ULI), missing upper or lower second premolars (PM2), and supernumerary teeth in the upper incisor region were determined. The twins were all enrolled in an ongoing study of dento-facial development being carried out at the dental schools in the universities of Adelaide and Melbourne. They ranged in age from four to 57 years, with most being teenagers. All were of European ancestry with no history of major medical disorders likely to be associated with missing or extra teeth. There were 132 male MZ pairs and 146 female MZ pairs.

**Table 1. Missing ULI and PM2 in MZ twin pairs (FDI notation used)**

Pair ID	Twin A	Twin B	Concordant/discordant
2m*	25	15	D (MI)†
27m	15, 25, 35, 45	15, 25, 35, 45	C
34f	12	–	D
49m	45	15, 25, 34, 35, 45	D
72f	15	–	D
73f	12, 22	22	D
76f	22	–	D
106f	45	–	D
118m	25	15, 25, 35, 45	D
128m	15, 25	25	D
155f	–	15	D
188f	22	12, 22	D
202m	–	35	D
216m	35	45	D (MI)
217m	45	–	D
262m	15, 25, 35, 45	–	D
263f	–	12	D
292f	22	12, 22, 45	D
313f	12	–	D
316f	12, 22	12, 22	C
360f	–	12	D
453m	45	–	D
577m	–	12	D
651m	12, 22	12, 22	C
Total 24			21D/3C

\*m=male, f=female.

†MI = mirror-imaged.

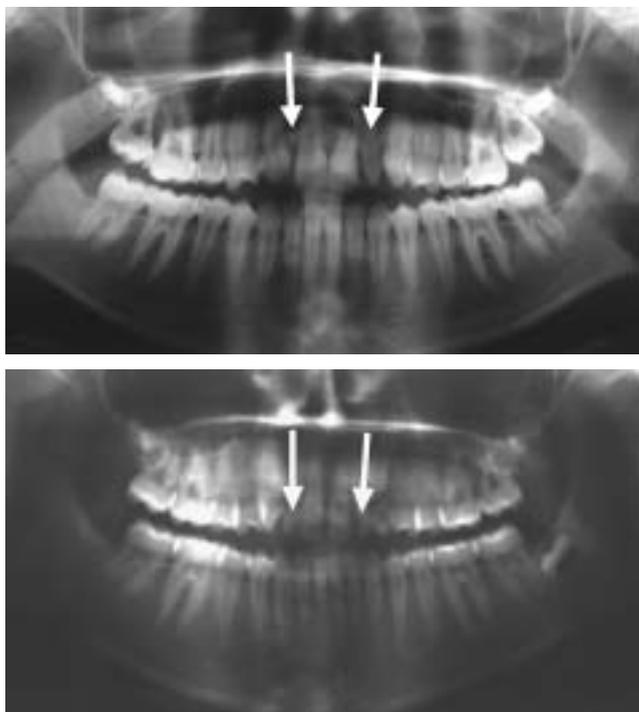
Zygosities of those twins examined in the 1980s were confirmed by comparisons of a number of genetic markers in the blood (ABO, Rh, Fy, Jk, MNS), together with several serum enzyme polymorphisms (GLO, ESD, PGM1, PGD, ACP, GPT, PGP, AK1) and protein polymorphisms (HP, C3, PI). Zygosities of those twins examined in the 1990s were 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. The probability of dizygosity, given concordance for all systems, was less than 1 per cent.

Data collection methods were approved by the Committee on the Ethics of Human Experimentation, University of Adelaide (Approval No. H/07/84A) and all participants were informed volunteers.

## RESULTS

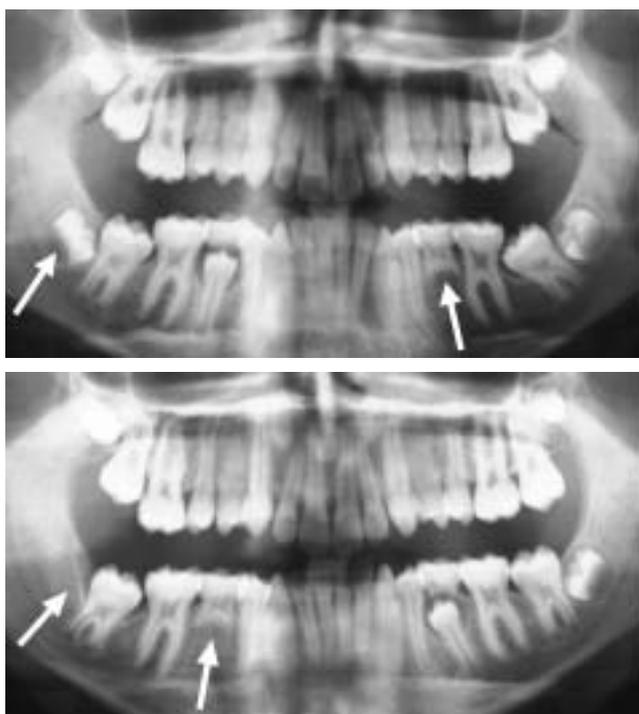
As shown in Table 1, there was evidence of at least one missing ULI or PM2 in 24 of the 278 MZ pairs examined (8.6 per cent), with 21 of these pairs showing discordant expression (87.5 per cent). Table 1 shows, for example, that Twin Pair #313 displayed discordant expression of ULI. In fact, Twin A had a missing right ULI and a peg-shaped left ULI whereas Twin B had two diminutive ULIs (Fig 1).

Twin Pair #216 also showed discordant expression for missing teeth, in this case involving PM2. Indeed, these twins displayed a mirror-imaged effect, with the lower left PM2 being missing in one twin and the lower right PM2 in the other (Fig 2).

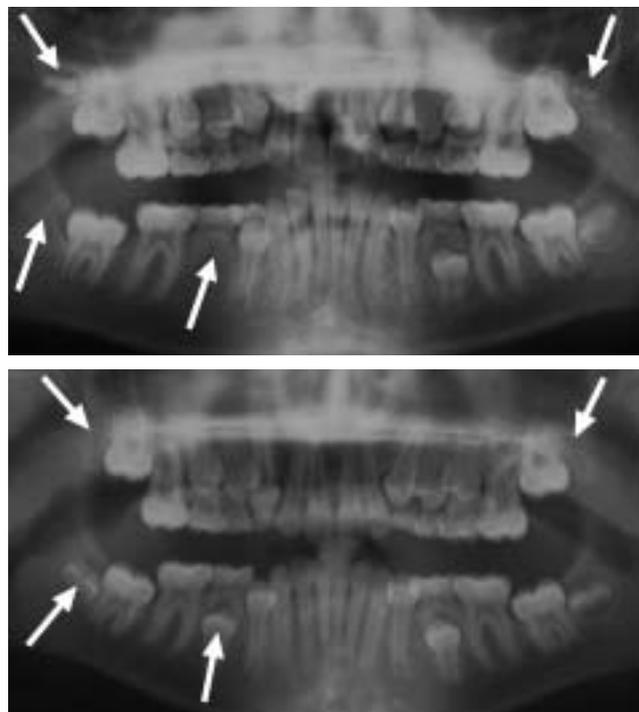


**Fig 1.** Panoramic radiographs of Twin Pair #313, a pair of MZ female twins aged 14 years. Twin A (top) has a missing right ULI and a peg-shaped left ULI (arrowed), whereas Twin B (bottom) has two diminutive ULIs (arrowed).

As another example, Twin Pair #453 also displayed discordant expression of PM2, with Twin A showing a missing lower right PM2 whereas the corresponding tooth was present in Twin B. These co-twins also



**Fig 2.** Panoramic radiographs of Twin Pair #216, a pair of MZ male twins aged 15 years, showing mirror-imaging for hypodontia of the lower PM2s. The lower left PM2 is missing in Twin A (top) (arrowed), whereas the lower right PM2 is missing in Twin B (bottom) (arrowed). In addition, a developing lower right third molar is evident in Twin A (arrowed) but not in Twin B (arrowed).



**Fig 3.** Panoramic radiographs of Twin Pair #453, a pair of MZ male twins aged 12.5 years. Twin A (top) has a missing lower right PM2 (arrowed), whereas the corresponding tooth is present in Twin B (bottom) (arrowed). In addition, there is no evidence of the lower right third molar in Twin A (arrowed) but the tooth is present in Twin B (arrowed). The upper third molars are evident in Twin A (arrowed) but not in Twin B (arrowed).

showed discordant expression of third molar development (Fig 3).

Nine of the MZ twin pairs (3.2 per cent) showed evidence of mesiodentes, with eight of these pairs showing discordant expression for the number of supernumeraries (88.9 per cent). Table 2 summarizes these data and shows that Twin Pair #491 was discordant for number of mesiodentes. Panoramic radiographs of this pair of twins are shown in Fig 4, with Twin A displaying one supernumerary and Twin B showing two.

## DISCUSSION

Keene<sup>13</sup> found that agenesis of teeth, other than third molars, was two to three times more frequent in a sample of 262 American twins than in the general

**Table 2.** Number of supernumerary teeth (mesiodentes) in MZ twin pairs

Pair ID	Twin A	Twin B	Concordant/discordant
186f	0	1	D
324m	2	0	D
328m	1	0	D
362f	0	1	D
491m	1	2	D
527m	1	2	D
630f	1	0	D
648m	0	1	D
807m	2	2	C
Total 9			8D/1C

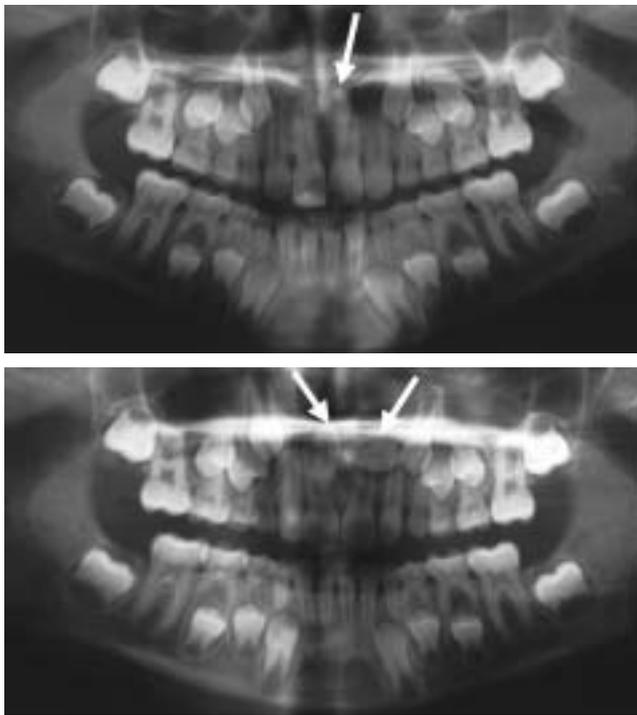


Fig 4. Panoramic radiographs of Twin Pair #491, a pair of MZ male twins aged 9.5 years. Twin A (top) has one mesiodens (arrowed), whereas Twin B (bottom) has two (arrowed).

population. Our estimate of 9 per cent for hypodontia involving ULI or PM2 is similar to the values of 8-9 per cent reported in other studies of twins<sup>14,15</sup> and falls at the higher end of the range of estimates for singleton populations.<sup>4</sup> However, no statistical comparisons were attempted and the differences could be due to differing sampling and recording methods. Mesiodentes have been reported to occur in 0.15 to 1.5 per cent of individuals,<sup>16,17</sup> so our estimate of 3 per cent for twins seems high. However, again this could be a sampling effect.

The high prevalence of discordant expression for tooth number in MZ twin pairs was a surprising finding in this study and contrasts with some previous reports. For example, Markovic<sup>14</sup> reported that most of the MZ twin pairs he examined were completely concordant for missing teeth. In contrast, Gravely and Johnson<sup>18</sup> found discordant expression in MZ co-twins in their studies of missing teeth and Kotsomitis *et al.*<sup>15</sup> also noted that most of the MZ twin pairs they examined with missing teeth displayed variable expression. Seddon *et al.*<sup>17</sup> concluded, after reviewing eight previous cases and one of their own, that mesiodentes were likely to be concordant in MZ twins with respect to number but they noted that minor variations in shape and orientation were common. Therefore, there is some uncertainty about the prevalence of discordant expression of dental features in MZ twins, although our findings certainly reinforce the point that using the term 'identical' when referring to 'monozygotic' twin pairs can be misleading. We have shown that most of the MZ twin pairs who displayed missing or extra teeth in our sample did not display

identical dentitions. What could be the possible explanation for these differences?

Martin *et al.*<sup>19</sup> have described a wide range of genetic and environmental influences to explain why MZ twin pairs might not be identical phenotypically. They list differential placental implantation and nutrition, as well as differential transplacental teratogens and infections as possible environmental effects. Post-zygotic genetic effects could include differential imprinting, post-zygotic non-disjunction and differential trinucleotide repeat expansion. It is possible that one or more of these genetic and environmental factors may contribute to the discordances we have noted in missing and extra teeth in MZ twin pairs but there are other possibilities.

Molenaar *et al.*<sup>20</sup> have referred to 'a third source of developmental differences', in addition to genetic and environmental factors, that they propose accounts for phenotypic differences in development. They argue that this third source consists of nonlinear epigenetic processes that can create variability at all phenotypic levels, both somatic and behavioural. They refer to a study of chaetae (bristles) in *Drosophila* reported by Mather and Jinks<sup>21</sup> in which variation in numbers of chaetae between right and left sides of inbred flies seemed to be attributable only to 'the vagaries of development . . . affecting the two sides of the thorax differently'. Furthermore, 91 per cent of the variation in chaetae numbers between flies was due to this same developmental variation. Molenaar *et al.* provide other examples of controlled studies in inbred animals that support the view that there is a third distinct and major source of phenotypic differences, in addition to genetic and environmental influences, that 'resides in the intrinsic indeterminacy' of the epigenetic processes underlying normal growth and development. They propose that these epigenetic influences result from autonomous developmental processes with 'emergent self-organizing properties'.

This concept of developmental systems with emergent self-organizing properties fits in nicely with what we now know about the molecular basis of tooth development. A series of interactions between epithelial and ectomesenchymal tissues, facilitated by the exchange of various signalling molecules leads to the initiation, morphogenesis and differentiation of developing teeth.<sup>22</sup> Furthermore, Jernvall and Jung<sup>23</sup> have described how the same genes are expressed and the same signalling molecules released to produce each of the cusps on a tooth. These genes appear to be highly conserved in an evolutionary sense and once odontogenesis has been initiated it tends to become a continuous self-organizing process. Crown patterns appear to evolve dynamically depending on the spatial and temporal expression of activating and inhibiting molecules produced by developing enamel knots.<sup>24</sup> Variations in dental phenotypes between species may therefore relate to regulation of certain conserved genes involved in tooth formation, while variations within

species may result from very minor variations in the timing of interactions between cells and in the positions of cells relative to each other. These are both examples of epigenetic mechanisms, one operating on the genome and the other acting at a local tissue level. In fact, researchers have now developed mathematical models to demonstrate how large morphological changes can be produced by small epigenetic events.<sup>25</sup>

The fact that the last teeth to develop within each series, that is, lateral incisors, premolars and third molars, tend to develop over longer periods of time than other teeth, and also tend to be missing most often, suggests that local epigenetic signals are likely to be susceptible to temporal variations. Indeed, radiographic studies have shown that late formation of tooth germs is one of the factors associated with congenital absence of other teeth.<sup>26</sup> So minor delays in the timing of developmental events in one region of the dentition may have flow-on effects on the process of tooth formation in other regions, with later-forming teeth being most susceptible to hypodontia. Extending this concept, accelerated development within the dentition affecting local signalling events may be associated with discordances in the number of supernumerary teeth in genetically susceptible MZ co-twins, but more studies are needed to support or refute this hypothesis.

Two examples of mirror-imaged effects involving PM2 development were noted in this study but, at present, we are unable to say whether they resulted from the chance effects of local epigenetic influences or, rather, reflected a more basic alteration in the determination of body symmetry associated with the twinning process. It has been suggested that mirror-imaging may be associated with a tendency for the zygote to divide later during embryogenesis, around the time when the body normally determines its symmetry.<sup>27</sup> To test this hypothesis, a systematic assessment of mirror-imaging is needed in monozygotic MZ twin pairs, who separate later during development, compared with dizygotic MZ twin pairs, who separate earlier. Given their bilateral arrangement, observations of a suite of facial and dental features should prove to be extremely valuable in this type of investigation.

## CONCLUSION

Our results show that differences in the expression of missing or extra teeth occur commonly between those MZ twin pairs whose genetic make-up predisposes them to display simple hypodontia or mesiodontes. We propose that minor variations in local epigenetic events in tooth-forming regions, possibly relating to spatial arrangements of cells or temporal events, may account for the distinct discordances in dental features observed in these MZ twin pairs.

## ACKNOWLEDGEMENTS

We wish to express our sincere thanks to the twins and their families who agreed to participate in this

study which was supported by Competing Epidemiological Grant 157904 from the NHMRC of Australia.

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