Journal of Dental Research
2017, Vol. 96(9) 1014–1019

DOI: 10.1177/0022034517711156
journals.sagepub.com/home/jdr

# Maternal Smoking during Pregnancy Is Associated with Offspring Hypodontia

**A.H. Al-Ani[1], J.S. Antoun[1], W.M. Thomson[1], T.R. Merriman[2], and M. Farella[1]**

**Abstract**

Little is known about environmental risk factors for hypodontia. The objective of this study was to investigate the association between hypodontia and common environmental risk factors, such as maternal smoking and alcohol and caffeine consumption during pregnancy. Eighty-nine hypodontia cases with 1 or more missing permanent lateral incisors and/or 1 or more missing premolars were enrolled in this clinic-based case-control study. Some 253 controls with no missing teeth were frequency matched to cases by age and sex. Hypodontia was diagnosed using panoramic radiographs. Sociodemographic data were collected from both the participants and their mothers, with maternal self-reported active and passive smoking, as well as alcohol and caffeine consumption during pregnancy, assessed by a questionnaire. Odds ratios (ORs) and their 95% confidence intervals (CIs) were calculated with logistic regression to assess the strength of association between risk factors and hypodontia. OR estimates were then adjusted for possible confounders, such as maternal age at delivery, sex and gestational age of the child, and household socioeconomic background. Significant associations were found between hypodontia and maternal cigarette use during pregnancy, as well as the number of cigarettes smoked per day. The consumption of 10 or more cigarettes per day during pregnancy was associated with greater odds of having a child with hypodontia (adjusted OR, 4.18; 95% CI, 1.48–11.80; $P = 0.007$). Observed associations between hypodontia, second-hand smoke, and alcohol and caffeine consumption were not statistically significant. Maternal smoking during pregnancy is associated with hypodontia. Larger samples and prospective observational study designs, however, are needed to investigate this association further.

**Keywords:** case-control studies, anodontia, tooth abnormalities, ethanol, tobacco, caffeine

## Introduction

Tooth agenesis is the most common dental developmental defect, with around 5% of people failing to develop at least 1 tooth (Polder et al. 2004). This prevalence estimate is considerably higher when third molars are included. Hypodontia, where fewer than 6 teeth are missing, is the most common form of tooth agenesis (Nunn et al. 2003; Yin and Bian 2015), with the mandibular and maxillary second premolars and maxillary lateral incisors being the most commonly missing teeth (Polder et al. 2004; Bailleul-Forestier et al. 2008). Agenesis occurs during the early stages of tooth development due to a breakdown of the communication pathways between the mesenchymal tissue and the overlying epithelium (Thesleff 2003), both of which originate from the neural crest.

The etiologic factors involved in tooth agenesis remain largely unknown, although it is well established that genetic variation plays a key role (Galluccio et al. 2012). Indeed, mutations in genes such as *MSX1, PAX9, AXIN2*, and *EDA* have been identified in familial forms of nonsyndromic hypodontia (Vastardis 2000; Lammi et al. 2004; Stockton 2008). Nevertheless, there is compelling evidence that conditions arising from defects in tooth morphogenesis (including hypodontia) are also caused by a complex combination of genetic and environmental factors (Krauss and Hong 2016).

Examples of environmental factors that can cause disruption to normal tooth development are trauma to the alveolar process or jaw, jaw surgery, or iatrogenic damage to the developing tooth germ from traumatic extraction of the overlying primary tooth (Grahnén 1956; Nunn et al. 2003). Infections during pregnancy (such as rubella) have been reported to associate with hypodontia in the developing child (Cameron and Sampson 1996; Parkin et al. 2009). It has also been reported that tooth agenesis is more common in children with thalidomide embryopathy (7.7%) than in those in the wider population (0.4%) (Axrup 1966), while a higher prevalence of tooth agenesis was observed among people exposed to dioxin in Seveso, Italy (Alaluusua et al. 2004). Similarly, the sensitivity of tooth development to childhood anticancer treatment in early infancy has also been implicated in hypodontia (Näsman

[1]Department of Oral Sciences, Faculty of Dentistry, University of Otago, Dunedin, New Zealand
[2]Department of Biochemistry, Faculty of Dentistry, University of Otago, Dunedin, New Zealand

**Corresponding Author:**
M. Farella, Discipline of Orthodontics, Department of Oral Sciences, Faculty of Dentistry, University of Otago, PO Box 647, Dunedin 9054, New Zealand.
Email: mauro.farella@otago.ac.nz

et al. 1997; Nunn et al. 2003; Dahllöf and Huggare 2004; Hölttä et al. 2005). Hence, it seems that a multitude of mechanical, biological, and chemical noxious stimuli can all disrupt the molecular pathways during the early stages of tooth development and ultimately result in tooth agenesis. Moreover, tooth development is regulated by inductive interactions between epithelial and mesenchymal cells, as in other organs in the craniofacial complex (Thesleff 2003). Consequently, the signaling molecular pathway disturbances in the developmental regulatory genes (e.g., transcription factors) that result in hypodontia appear to be similar to those that cause craniofacial defects (Thesleff 1998).

Smoking and alcohol use are recognized human teratogens and risk factors for developmental disorders, including craniofacial disorders (Gilbert-Barness 2010; Mead and Sarkar 2014). Maternal smoking, for instance, has long been associated with a higher risk of birth defects such as cleft lip and/or palate (CL/P), with the odds of having children with CL/P among mothers who smoke almost 1.3 times those who do not (Little et al. 2004; Shi et al. 2007; Dixon et al. 2011). Alcohol consumption is associated with fetal alcohol spectrum disorders and characteristic craniofacial features (Krauss and Hong 2016). Moreover, alcohol has been associated with holoprosencephaly (HPE) (Krauss and Hong 2016), with fetal alcohol exposure inducing craniofacial anomalies and strain-dependent HPE in mice (Hong and Krauss 2012). Exposure to maternal alcohol consumption has also been proposed as a risk factor for the development of CL/P (Mossey and Little 2009), although links to alcohol consumption have yet to be confirmed (Dixon et al. 2011).

To our knowledge, previous studies have not investigated the association between nonsyndromic hypodontia and environmental factors such as maternal smoking and alcohol consumption during pregnancy. Tobacco smoking and alcohol consumption are risk factors for craniofacial birth defects (Dixon et al. 2011), while their adverse effects on reproductive health are well known. Accordingly, it is plausible that an association exists between nonsyndromic tooth agenesis and these risk factors.

The aims of this case-control study were to test the hypotheses that nonsyndromic hypodontia is positively associated with exposure to smoking, alcohol, or caffeine during pregnancy.

## Materials and Methods

### *Participants*

This research report conforms to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines for case-control studies (von Elm et al. 2008). All study procedures were approved by the University of Otago Human Ethics Committee (H14/080), and all participants provided written informed consent.

Participants were recruited from a pool of new patients or patients undergoing treatment in the orthodontic clinic at the University of Otago (Dunedin, New Zealand) from August 2014 to December 2015.

All of these patients were screened for eligibility according to the following inclusion criteria: age ≥9 y, availability of a preexisting good-quality baseline panoramic radiograph (OPG), and willingness to participate and provide informed consent to take part in the study. Hypodontia cases were defined as patients with 1 or more missing permanent teeth, except third molars, whereas controls were defined as patients without any missing teeth, except third molars. The same exclusion criteria were applied to both cases and controls and included missing teeth due to previous trauma, extraction, or craniofacial syndromes. Ongoing or previous orthodontic treatment did not preclude participation in this study. Eligible participants were offered a free movie voucher as an incentive for participating in the study.

The recruitment of cases involved clinical and radiographic assessment of eligible patients. Existing pretreatment OPGs (not older than 12 mo) were examined by 1 investigator (A.H.A.-A.) to identify missing permanent tooth/teeth, excluding the third molars. A tooth was recorded as congenitally missing if no evidence of the tooth or its developmental crypt was found on the radiograph. Treatment records were also checked to confirm that the missing tooth/teeth had not been extracted. The use of OPGs for the diagnosis of hypodontia and other dental anomalies has been considered a valid approach (Schalk-van der Weide et al. 1992).

The recruitment of controls commenced once 75% of the cases were recruited, to ensure that the 2 groups were comparable for age and sex (frequency matching). Preexisting OPGs were again used to assess whether any permanent teeth (excluding the third molars) were missing. Controls were recruited from the same pool of orthodontic patients as the cases and were enrolled in the study in the same manner as the cases.

We aimed to recruit study participants using a 1:3 case-to-control ratio. Setting type I error at 0.05 and using a prevalence rate for maternal smoking in New Zealand of 24% (Mitchell et al. 2002), we estimated that 38 hypodontia cases were needed to detect an odds ratio ≥3.0, 56 cases were needed to detect an odds ratio ≥2.5, and 101 cases were needed to detect an odds ratio ≥2.0. Post hoc power analyses were carried out as needed. Sample size estimation was also based on the minor allele frequencies of a number of candidate genes investigated (MSX1, PAX9, AXIN2, and EDA) for a parallel study. The findings of that study are not included in this report.

The selected case sample comprised 89 patients with 1 or more missing permanent lateral incisors and/or 1 or more missing permanent premolars. These cases were frequency matched by age and sex to 253 controls with no missing teeth.

Selected cases were contacted initially by post and invited to participate in the study. This was followed by a phone call during which the objectives of the study were further discussed, and an appointment was arranged if the individual was willing to participate in the study. Information sheets outlining the purpose, nature, and design of the study were provided to each participant during this appointment, and both enrollment and data collection commenced if the participant/parents provided informed consent.

**Table 1.** Comparison of Sociodemographic Characteristics between Hypodontia Cases and Controls.

| Characteristic | Group | | |
|---|---|---|---|
| | Cases ($n$ = 89) | Controls ($n$ = 253) | $P$ Value[a] |
| Age, mean (SD), y | 15.9 (5.1) | 16.9 (7.9) | |
| Sex, $n$ (%) | | | |
| Female | 51 (57.3) | 153 (60.5) | 0.600 |
| Male | 38 (42.7) | 100 (39.5) | |
| Gestation age, $n$ (%)[b] | | | |
| Early | 26 (29.2) | 76 (30.0) | 0.833 |
| Full term | 28 (31.5) | 83 (32.9) | |
| Late | 25 (28.0) | 62 (24.5) | |
| Mothers, $n$ (%) | | | |
| Ethnicity[c] | | | |
| NZ European | 67 (75.3) | 191 (75.5) | 0.803 |
| NZ Māori | 2 (2.2) | 4 (1.6) | |
| Other | 9 (10.1) | 20 (7.9) | |
| Socioeconomic status[d] | | | |
| High (1 to 2) | 27 (30.3) | 67 (26.5) | 0.517 |
| Medium (3 to 4) | 39 (43.8) | 110 (43.5) | |
| Low (5 to 6) | 5 (5.6) | 23 (9.1) | |
| Age at delivery[e] | | | |
| <20 y | 3 (3.4) | 9 (3.6) | 0.491 |
| 20 to 30 y | 27 (30.3) | 74 (29.2) | |
| 30 to 40 y | 42 (47.2) | 105 (41.5) | |
| >40 y | 4 (4.5) | 23 (9.1) | |

Percentages are column percentages. NZ, New Zealand.
[a]Chi-square test.
[b]Missing data: 10 (11.3%) cases, 32 (12.6%) controls.
[c]Missing data: 11 (12.4%) cases, 38 (15.0%) controls.
[d]Missing data: 18 (20.2%) cases, 53 (20.9%) controls.
[e]Missing data: 13 (14.6%) cases, 42 (16.6%) controls.

## *Exposure and Outcome Variables*

Study participants and their mothers were asked to attend a one-off appointment to complete the study questionnaires, which sought information about the participant's age, sex, and ethnicity.

Information on maternal exposures to risk factors during pregnancy was collected using maternal self-report. Information also was collected about maternal sociodemographic characteristics, smoking habits (including exposure to second-hand smoking), the number of cigarettes smoked per day, and alcohol consumption assessed as glasses per week. We considered mothers as exposed to active smoking if they had smoked for at least 1 mo during pregnancy.

Caffeine consumption during pregnancy was also collected and recorded on a binary scale (yes/no). In addition, information about gestational age was collected, with this grouped into 3 ordinal categories: "early" if the baby was born before 37 wk, "full term" if born at 37 to 40 wk, and "late" if born after 40 wk. Questionnaires were posted out with a prepaid return envelope to the mothers who were unable to attend the appointment.

The outcome variable hypodontia was in the form of a binary variable (yes/no) indicating whether or not 1 or more teeth were missing, and when this was the case, the affected teeth were recorded using a standard 2-digit notation (ISO 3950).

All questionnaire data were extracted by a research assistant who was blind to the case-control status of patients and their mothers.

## *Statistical Analysis*

Data were analyzed using conventional descriptive methods. Univariate and multivariate regression procedures were applied to estimate the associations between maternal smoking and alcohol exposure during pregnancy and nonsyndromic hypodontia. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated using binary logistic regression (univariate and bivariate). Smoking severity was coded as an ordinal variable with 3 levels and entered into the model using 2 dummy variables. Alcohol and caffeine consumptions were coded as binary variables (0, 1).

The regression models were adjusted for the following potential confounders: maternal age at delivery (continuous covariate), female sex (binary variable) and gestational age of the child (3 levels: early, on time, late), and household socioeconomic background (three levels: high, medium, low). Household socioeconomic status (SES) was determined using the New Zealand Socio-economic Index 2006 (Milne et al. 2013) and scored on an ordinal scale (low, medium, high). Socioeconomic status and gestational age were entered in the binary logistic regression model using dummy variables. Statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS v22.0; SPSS, Inc.).

# Results

Most of the cases ($n$ = 47, 52.8%) had agenesis of 1 or more permanent premolars, while approximately one-third had agenesis of the permanent lateral incisors ($n$ = 29, 32.6%). A combination of premolar and lateral incisor agenesis was observed in 12 cases (13.5%), while 1 individual (1.1%) was missing these teeth in combination with the lower second molars.

The sociodemographic characteristics of the study sample are summarized in Table 1. Most of the cases and controls were female. There were no significant differences between cases and control in any of the sociodemographic characteristics.

Maternal exposures to risk factors during pregnancy are summarized in Table 2. The proportion of mothers reporting smoking in the hypodontia group was more than twice as high as that of the control group. The frequency of smoking (i.e., the number of cigarettes per day) also differed between the groups and showed a biological gradient, with 50% ($n$ = 9) of the mothers reporting smoking heavily during pregnancy (>10 cigarettes per day) having children with hypodontia. More than half of the smoking mothers (23/41; 56.1%) smoked for the entire pregnancy time. Maternal exposure to second-hand smoking did not differ between the 2 groups. A higher proportion of mothers of hypodontia individuals than controls reported drinking more than 1 glass of alcohol per week during pregnancy, although this difference was not statistically significant. There was no difference between cases and controls in maternal caffeine consumption during pregnancy.

Table 3 presents the unadjusted and adjusted odds ratios for hypodontia. Heavy maternal smoking during pregnancy was associated with a 4-fold higher risk of having a child with hypodontia. Maternal alcohol consumption during pregnancy was not associated with hypodontia in the child. This was also true for gestational age and maternal consumption of caffeine during pregnancy. In addition, the children of older mothers and lower SES mothers were no more at risk.

**Table 2.** Maternal Smoking Habits and Consumption of Alcohol and Caffeine during Pregnancy.

| Characteristic | Group, *n* (%) | | *P* Value[a] |
|---|---|---|---|
| | Cases (*n* = 89) | Controls (*n* = 253) | |
| Smoking habits[b] | | | |
| No | 61 (68.5) | 199 (78.7) | 0.004[c] |
| Yes | 18 (20.2) | 22 (8.7) | |
| Number of cigarettes/day[b] | | | |
| 0 | 61 (68.5) | 199 (78.7) | 0.031[d] |
| 1 to 9 | 9 (10.1) | 13 (5.1) | |
| >10 | 9 (10.1) | 9 (3.6) | |
| Second-hand smoking[b] | | | |
| No | 65 (73.0) | 175 (69.2) | 0.555 |
| Yes | 14 (15.7) | 46 (18.2) | |
| Alcohol consumption[b] | | | |
| No | 64 (71.9) | 195 (77.1) | 0.109 |
| > 1 glass/week | 15 (16.8) | 26 (10.3) | |
| Caffeine consumption[b] | | | |
| No | 29 (32.6) | 76 (30.0) | 0.711 |
| Yes | 50 (56.2) | 145 (57.3) | |

Percentages are column percentages.
[a]Chi-square test.
[b]Missing data: 10 (11.3%) cases, 32 (12.6%) controls.
[c]Still significant after Bonferroni correction (adjusted *P* = 0.0198).
[d]Not significant after Bonferroni correction (adjusted *P* = 0.155).

## Discussion

This study examined the association between nonsyndromic hypodontia and common environmental factors such as exposure to maternal smoking and alcohol and caffeine consumption during pregnancy. The findings suggest that 1) heavy maternal cigarette smoking during pregnancy is significantly associated with hypodontia, and 2) the strength of this association may suggest a biological gradient, with the consumption of 10 or more cigarettes per day during pregnancy associated with greater odds of having a child with hypodontia. The lack of similar studies makes it difficult to compare these findings with previous literature. Instead, this work provides new evidence to support the hypothesis that maternal smoking during pregnancy is a risk factor for having a child with hypodontia.

The etiology of hypodontia has often been attributed to both genetic and environmental factors (Parkin et al. 2009); however, an association between this developmental condition and common environmental risk factors has not been previously reported. Investigations into this aspect of tooth agenesis are of utmost importance, since environmental risk factors may present the best possibility for developing preventive measures.

Craniofacial bones, cartilage, nerves, and connective tissues all originate from neural crest cells. Specific developmental cascades are therefore common to the morphogenesis of both teeth and some craniofacial structures (Matalova et al. 2008), and indeed, several syndromes involving hypodontia often exhibit various dysplasias and clefts. Environmental factors have long been known to be associated with a higher risk of some of these craniofacial anomalies. Factors such as trauma, infections, and toxins have been implicated (Brook 2009). Neural crest cells are extremely sensitive to high levels of oxidative stress that can arise due to both genetic and environmental factors (Morgan et al. 2008; Sakai et al. 2016). It is generally accepted that oxidative stress, in the form of smoking, for example (van der Vaart et al. 2004), plays a central role in the pathogenesis of neural crest cells disorders and the etiology of craniofacial anomalies. In fact, maternal smoking and alcohol consumption during pregnancy have long been implicated with a higher risk of craniofacial deformities such as CL/P (Chung et al. 2000; Mossey and Little 2009; Dixon et al. 2011; DeRoo et al. 2016; McKinney et al. 2016). Despite the common genetic pathways shared between CL/P and hypodontia and the fact that these environmental exposures are relatively common, no study to date has tested a possible association between hypodontia and maternal smoking and alcohol use during pregnancy.

Apart from its novelty, the study has several strengths. First, adjustment for confounding factors associated with cigarette smoking, such as alcohol consumption (Chung et al. 2000), was made. All individuals with any other associated congenital anomalies and/or syndromes (such as CL/P) were also excluded to avoid confounding of the association between cigarette smoking and hypodontia. Second, the findings also hinted at a biological gradient with high odds of having newborns with hypodontia with greater maternal cigarette smoking during pregnancy. This is important, as a biological gradient effect is known to be a criterion of causation of a disease by an exposure (Hill 1965). Third, risk factors other than smoking were also investigated, such as maternal consumption of alcohol and caffeine during pregnancy. Maternal consumption of alcohol during pregnancy is known to be associated with having children affected by fetal alcohol syndrome or craniofacial anomalies (DeRoo et al. 2016; Krauss and Hong 2016). Interestingly, no association between these environmental factors and hypodontia was observed. These negative findings could be due to an insufficient number of study participants. Indeed, post hoc power analysis for testing the association between maternal alcohol and caffeine consumption and hypodontia ranged from 37.9% to 88.2%. Nonetheless, it is also possible that alcohol consumption was underreported as a result of the stigma surrounding maternal alcohol consumption during pregnancy. For caffeine consumption, our findings were consistent with the existing literature, where caffeine use has repeatedly been shown to have no association with congenital anomalies or the overall health of newborns (Browne 2006).

The present study has several potential limitations. First, the self-reported exposure data could be influenced by recall bias, and this may have affected their validity. Second, the use of healthy individuals with no missing teeth in the control group

**Table 3.** Unadjusted and Adjusted Odds Ratios for Hypodontia.

| | Unadjusted | | Adjusted[a] | |
|---|---|---|---|---|
| | Odds Ratio (95% CI) | *P* Value | Odds Ratio (95% CI) | *P* Value |
| Number of cigarettes/day | | | | |
| 0 | 1.00 | | 1.00 | |
| 1 to 9 | 2.26 (0.92–5.54) | 0.075 | 2.05 (0.73–5.75) | 0.232 |
| >10 | 3.26 (1.24–8.58) | 0.017 | 4.18 (1.49–11.80) | 0.007 |
| Alcohol consumption | 1.76 (0.88–3.52) | 0.109 | 1.84 (0.83–4.08) | 0.132 |
| Caffeine consumption | 0.90 (0.53–1.54) | 0.711 | 0.89 (0.48–1.64) | 0.700 |
| Maternal age at delivery | 1.01 (0.96–1.05) | 0.800 | 1.01 (0.96–1.07) | 0.622 |
| Female | 1.14 (0.70–1.86) | 0.600 | 1.32 (0.73–2.39) | 0.358 |
| Socioeconomic status | | | | |
| High (1 to 2) | 1.00 | | 1.00 | |
| Medium (3 to 4) | 0.88 (0.49–1.57) | 0.664 | 0.84 (0.45–1.56) | 0.575 |
| Low (5 to 6) | 0.54 (0.19–1.57) | 0.256 | 0.49 (0.16–1.56) | 0.228 |
| Gestational age | | | | |
| On time | 1.00 | | 1.00 | |
| Early | 1.01 (0.55–1.88) | 0.965 | 0.84 (0.40–1.76) | 0.646 |
| Late | 1.20 (0.64–2.25) | 0.580 | 1.15 (0.59–2.24) | 0.692 |

CI, confidence interval.
[a]All variables entered into the model. Nagelkerke's $R^2 = 0.062$.

introduces the risk of differential recall bias, since cases and their mothers may recall their exposure more vividly than the healthy controls, because they are more affected by the deformity of interest and have had longer to reflect on possible causes. As a result, cases may report greater exposure, and this would in turn inflate the odds ratio and bias against the null hypothesis. On the other hand, guilt or social desirability bias in mothers of hypodontia cases may have prompted them to underreport the smoking or alcohol exposures during pregnancy, resulting in an underestimation of the odds ratio and so favoring the null hypothesis. These limitations could be addressed by allocating individuals with other congenital anomalies that have not been found to have an association with smoking exposure to a second group of controls. This would potentially minimize recall bias, since mothers of both cases and controls should recall their smoking exposure similarly. Alternatively, a prospective study design would also address this limitation, given that the outcome is not very rare. In terms of smoking history, the main limitation in the questionnaire was that it did not collect details on the temporality and duration of smoking. Third, the study involved exploration of the associations in a clinic-based sample. These associations may not be generalizable to the whole community because the clinical cases may have different characteristics from community cases. Fourth, the sample size was small and lacked a replication sample. Nonetheless, the sample size used showed statistical significance, even after correcting for multiple comparisons. Accordingly, this study should be replicated in a larger center. Finally, there may have been overdiagnosis of hypodontia in patients with delayed tooth development. This was largely prevented, however, by the age cutoff (9 y), since delays after that age, although still possible, are rare.

## Conclusion

Our findings provide evidence of an association between hypodontia and maternal smoking. There is a suggestion of a biological gradient effect to this association. Direct damage of neural crest cells from oxidative stress agents, such as smoking, might help to explain a causal relationship between hypodontia and smoking. Larger samples and prospective observational study designs are needed to investigate this association further.

### Author Contributions

A.H. Al-Ani, contributed to conception, design, data acquisition, analysis, and interpretation, drafted the manuscript; J.S. Antoun, contributed to conception, design, data acquisition, and interpretation, critically revised the manuscript; W.M. Thomson, contributed to design, data analysis, and interpretation, critically revised the manuscript; T.R. Merriman contributed to design and data interpretation, critically revised the manuscript; M. Farella contributed to conception, design, data analysis, and interpretation, critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

### Acknowledgments

This work was supported by grants from the New Zealand Dental Association and the Health Research Council of New Zealand, as well as a Fuller grant from the University of Otago Faculty of Dentistry. We also gratefully acknowledge the contributions of the following individuals: Ms. Cindy Mullens, Ms. Chantelle Rossouw, Ms. Ruth Topless, and the staff from Merriman Laboratory. The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

### References

Alaluusua S, Calderara P, Gerthoux PM, Lukinmaa P-L, Kovero O, Needham L, Patterson DG, Tuomisto J, Mocarelli P. 2004. Developmental dental aberrations after the dioxin accident in Seveso. Environ Health Perspect. 112(13):1313–1318.

Axrup K, D'Avignon M, Hellgren K, Herikson CO, Juhlin IM, Larsson KS, Persson GE, Welander E. 1966. Children with thalidomide embryopathy: odontological observations and aspects. Acta Odontol Scand. 24(1):3–21.

Bailleul-Forestier I, Molla M, Verloes A, Berdal A. 2008. The genetic basis of inherited anomalies of the teeth. Part 1: Clinical and molecular aspects of non-syndromic dental disorders. Eur J Med Genet. 51(4):273–291.

Brook AH. 2009. Multilevel complex interactions between genetic, epigenetic and environmental factors in the aetiology of anomalies of dental development. Arch Oral Biol. 54(Suppl 1):S3–S17.

Browne ML. 2006. Maternal exposure to caffeine and risk of congenital anomalies: a systematic review. Epidemiology. 17(3):324–331.

Cameron J, Sampson WJ. 1996. Hypodontia of the permanent dentition: case reports. Aust Dent J. 41(1):1–5.

Chung KC, Kowalski CP, Kim HM, Buchman SR. 2000. Maternal cigarette smoking during pregnancy and the risk of having a child with cleft lip/palate. Plast Reconstr Surg. 105(2):485–491.

Dahllöf G, Huggare J. 2004. Orthodontic considerations in the pediatric cancer patient: a review. Semin Orthod. 10(4):266–276.

DeRoo LA, Wilcox AJ, Lie RT, Romitti PA, Pedersen DA, Munger RG, Moreno Uribe LM, Wehby GL. 2016. Maternal alcohol binge-drinking in the first trimester and the risk of orofacial clefts in offspring: a large population-based pooling study. Eur J Epidemiol. 31(10):1021–1034.

Dixon MJ, Marazita ML, Beaty TH, Murray JC. 2011. Cleft lip and palate: understanding genetic and environmental influences. Nat Genet. 12(3):167–178.

Galluccio G, Castellano M, La Monaca C. 2012. Genetic basis of non-syndromic anomalies of human tooth number. Arch Oral Biol. 57(7):918–930.

Gilbert-Barness E. 2010. Teratogenic causes of malformations. Ann Clin Lab Sci. 40(2):99–114.

Grahnén H. 1956. Hypodontia in the permanent dentition: a clinical and genetical investigation. Odontol Revy. 7(Suppl 3):1–100.

Hill AB. 1965. The environment and disease: association or causation? Proc R Soc Med. 58:295–300.

Hölttä P, Alaluusua S, Saarinen-Pihkala UM, Peltola J, Hovi L. 2005. Agenesis and microdontia of permanent teeth as late adverse effects after stem cell transplantation in young children. Cancer. 103(1):181–190.

Hong M, Krauss RS. 2012. Cdon mutation and fetal ethanol exposure synergize to produce midline signaling defects and holoprosencephaly spectrum disorders in mice. PLoS Genet. 8(10):e1002999.

Krauss RS, Hong M. 2016. Gene-environment interactions and the etiology of birth defects. Curr Top Dev Biol. 116:569–580.

Lammi L, Arte S, Somer M, Jarvinen H, Lahermo P, Thesleff I, Pirinen S, Nieminen P. 2004. Mutations in AXIN2 cause familial tooth agenesis and predispose to colorectal cancer. Am J Hum Genet. 74(5):1043–1050.

Little J, Cardy A, Munger RG. 2004. Tobacco smoking and oral clefts: a meta-analysis. Bull World Health Organ. 82(3):213–218.

Matalova E, Fleischmannova J, Sharpe PT, Tucker AS. 2008. Tooth agenesis: from molecular genetics to molecular dentistry. J Dent Res. 87(7):617–623.

McKinney CM, Pisek A, Chowchuen B, DeRouen T, Muktabhant B, Pradubwong S, Yeung C, Pitiphat W. 2016. Case-control study of nutritional and environmental factors and the risk of oral clefts in Thailand. Birth Defects Res A Clin Mol Teratol. 106(7):624–632.

Mead EA, Sarkar DK. 2014. Fetal alcohol spectrum disorders and their transmission through genetic and epigenetic mechanisms. Front Genet. 5:154.

Milne B, Byun U, Lee A. 2013. New Zealand socio-economic index 2006. Wellington: Statistics New Zealand.

Mitchell EA, Thompson JM, Robinson E, Wild CJ, Becroft DM, Clark PM, Glavish N, Pattison NS, Pryor JE. 2002. Smoking, nicotine and tar and risk of small for gestational age babies. Acta Paediatr. 91(3):323–328.

Morgan SC, Relaix F, Sandell LL, Loeken MR. 2008. Oxidative stress during diabetic pregnancy disrupts cardiac neural crest migration and causes outflow tract defects. Birth Defects Res A Clin Mol Teratol. 82(6):453–463.

Mossey P, Little J. 2009. Addressing the challenges of cleft lip and palate research in India. Indian J Plast Surg. 42(Suppl):S9–S18.

Näsman M, Forsberg C, Dahllöf G. 1997. Long-term dental development in children after treatment for malignant disease. Eur J Orthod. 19(2):151–159.

Nunn JH, Carter NE, Gillgrass TJ, Hobson RS, Jepson NJ, Meechan JG, Nohl FS. 2003. The interdisciplinary management of hypodontia: background and role of paediatric dentistry. Br Dent J. 194(5):245–251.

Parkin N, Elcock C, Smith RN, Griffin RC, Brook AH. 2009. The aetiology of hypodontia: the prevalence, severity and location of hypodontia within families. Arch Oral Biol. 54(Suppl 1):S52–S56.

Polder BJ, Van't Hof MA, Van der Linden FP, Kuijpers-Jagtman AM. 2004. A meta-analysis of the prevalence of dental agenesis of permanent teeth. Community Dent Oral Epidemiol. 32(3):217–226.

Sakai D, Dixon J, Achilleos A, Dixon M, Trainor PA. 2016. Prevention of Treacher Collins syndrome craniofacial anomalies in mouse models via maternal antioxidant supplementation. Nat Commun. 7:10328.

Schalk-van der Weide Y, Steen WH, Bosman F. 1992. Distribution of missing teeth and tooth morphology in patients with oligodontia. ASDC J Dent Child. 59(2):133–140.

Shi M, Christensen K, Weinberg CR, Romitti P, Bathum L, Lozada A, Morris RW, Lovett M, Murray JC. 2007. Orofacial cleft risk is increased with maternal smoking and specific detoxification-gene variants. Am J Hum Genet. 80(1):76–90.

Stockton DW, Das P, Goldenberg M, D'Souza RN, Patel PI. 2008. Mutation of PAX9 is associated with oligodontia. Nat Genet. 24(1):18–19.

Thesleff I. 1998. The genetic basis of normal and abnormal craniofacial development. Acta Odontol Scand. 56(6):321–325.

Thesleff I. 2003. Developmental biology and building a tooth. Quintessence Int. 34(8):613–620.

van der Vaart H, Postma DS, Timens W, ten Hacken NH. 2004. Acute effects of cigarette smoke on inflammation and oxidative stress: a review. Thorax. 59(8):713–721.

Vastardis H. 2000. The genetics of human tooth agenesis: new discoveries for understanding dental anomalies. Am J Orthod Dentofacial Orthop. 117(6):650–656.

von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP, STROBE Initiative. 2008. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. J Clin Epidemiol. 61(4):344–349.

Yin W, Bian Z. 2015. The gene network underlying hypodontia. J Dent Res. 94(7):878–885.