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Etiology of Hypomineralized Second Primary Molars: A Prospective Twin Study

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Abstract

The etiology of hypomineralized second primary molars (HSPM) is unclear, but genetic and environmental factors have been proposed. The aim of this study was to investigate the relative contribution of genes and environment to the etiology of HSPM and to identify potential environmental risk factors in a longitudinal twin cohort. Children from twin pregnancies ($N = 250$) were recruited antenatally, and detailed demographic, health, and phenotypic data were collected at recruitment, 24- and 36-wk gestation, birth, and 18 mo of age. 25-Hydroxyvitamin D was quantified for mothers at 28-wk gestation and infants at birth. Dental examinations were conducted on the twins at 6 y of age to determine the presence, severity, and extent of HSPM per standardized criteria. To investigate associations of environmental risk factors with HSPM, multiple logistic regression models were fitted with generalized estimating equations to adjust for twin correlation. Within- and between-pair analyses were performed for unshared continuous variables: birthweight and birth 25-hydroxyvitamin D. Twin-twin concordance for monozygotic (MZ) and dizygotic (DZ) pairs was calculated and compared after adjusting for identified risk factors. A total of 344 twins underwent the 6-y-old dental assessment; HSPM occurred in 68 (19.8%). After adjusting for potential confounders, vitamin D levels at birth, infantile eczema, dizygosity, in vitro fertilization, socioeconomic position, and maternal smoking beyond the first trimester of pregnancy demonstrated the strongest associations with HSPM. Overall concordance for HSPM was 0.47 (95% CI, 0.32 to 0.62) with weak evidence ($P = 0.078$) of higher concordance in MZ twins (0.63; 95% CI, 0.38 to 0.89) as compared with DZ twins (0.41; 95% CI, 0.24 to 0.58). After adjusting for known risk factors, there was no evidence ($P = 0.172$) for an additive genetic influence. These findings suggest that shared and unshared environmental factors, such as maternal smoking later in pregnancy and infantile eczema, are important in the etiology of HSPM.

Keywords: dental enamel, twins, tooth, deciduous, prospective studies, risk factors

Introduction

Hypomineralized second primary molars (HSPM) describe demarcated qualitative defects of enamel of systematic origin affecting ≥1 second primary molars (Elfrink et al. 2008). The condition is a risk factor for molar-incisor hypomineralization (MIH), sharing similar clinical presentation, structural properties, and putative etiology (Elfrink et al. 2012; Mittal and Sharma 2015; Negre-Barber et al. 2016). The prevalence of HSPM is between 4% and 14.5% (Elfrink et al. 2008; Ghanim et al. 2013; Mittal and Sharma 2015; Temilola et al. 2015; Negre-Barber et al. 2016; Owen et al. 2018). In addition to serving as a useful indicator of MIH risk, HSPM contribute to caries risk among young children (Elfrink et al. 2010).

Although the causes of MIH and HSPM are currently unknown, a combination of environmental factors from the prenatal and early life period and genetic and epigenetic factors is thought to contribute (Vieira and Kup 2016; Teixeira et al. 2018). Conclusions from observational studies have been hampered by the lack of prospective exposure data and standardized outcome measurement (Silva et al. 2016).

Twin studies have traditionally been used to explore the contribution of genes and environment to the etiology of complex traits. Monozygotic (MZ) twins share 100% of their genetic makeup, whereas dizygotic (DZ) twins share 50% (on

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A supplemental appendix to this article is available online.

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average). Comparison of concordance in MZ and DZ pairs can help determine the influence of shared, nonshared, and genetic factors to the variation in risk of HSPM (Neale et al. 1994). A higher concordance for MIH in MZ twins as compared with DZ twins was recently reported, suggesting a genetic influence, but no analogous data are available for HSPM (Teixeira et al. 2018).

The aim of this study was to use a longitudinal twin cohort to investigate the relative contribution of genes and environment to the etiology of HSPM, to identify potential environmental risk factors, and to determine the influence of these factors on twin concordance.

Materials and Methods

Peri/postnatal Epigenetic Twins Study

Established in 2007, the Peri/postnatal Epigenetic Twins Study is a longitudinal study of a birth cohort of 250 mothers and their twin children. A detailed description of the cohort was reported previously (Saffery et al. 2012). Women pregnant with twins were recruited midgestation. Questionnaires regarding maternal nutrition, illness, medication use, alcohol intake, and smoking were collected at 3 time points during the pregnancy. Maternal stress was measured with a validated questionnaire (Cohen et al. 1983). The socioeconomic status (SES) of participants at birth was obtained by linking to the Index of Relative Socio-economic Disadvantage, which is one of the Socio-economic Indexes for Areas (SEIFA) developed by the Australian Bureau of Statistics (2006) based on census data via residential postcodes. The children were examined (and hospital records accessed) in the immediate postnatal period to obtain anthropometric data as well as details regarding the delivery and health of the newborns. Zygosity was determined according to chorionicity, sex, and genetics. All different-sex twins were presumed to be DZ, but zygosity was determined by a 12-marker microsatellite test with DNA from cord and/or buccal samples for all same-sex twin pairs (Becker et al. 1997).

A total of 244 twin pairs were reviewed at age 18 mo, and data about feeding, illness, hospitalization, medication use were collected from parents. From 2014 to 2016, the children (now aged 6 y) were examined again, and dental examinations were performed in addition to collection of data about health and development (Appendix).

Maternal 25-hydroxyvitamin D levels were determined from serum collected from mothers at 28 wk of gestation. Child 25-hydroxyvitamin D levels at birth were determined from serum and plasma from cord blood. For all samples, 25-hydroxyvitamin D levels were determined with the LIAISON 25-OH Vitamin D Total kit (DiaSorin). A subset of newborn serum samples were analyzed in 2011, and the remaining serum and plasma samples were analyzed in 2017. Batching effects between measurements taken from serum in 2017 (used as the reference) and those measured from serum in 2011 or plasma in 2017 were explored and addressed (see Appendix for details regarding the statistical approach used to test and adjust for batching effects).

Ethics approval was obtained from the Royal Children's Hospital Human Research Ethics Committee (33174 A), and informed consent was obtained from parents. This observational study conforms to the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines.

Dental Examinations

Dental examinations were performed on-site at a research facility or, for participants unable to travel, at home. The examinations were performed by 2 trained and calibrated oral health professionals (M.J.S., P.L.; see Appendix). On-site examinations were performed with an overhead light and with the child reclined on a clinical examination bed. During home visits ($n = 66$), examinations were performed with a headlight and with the child supine (on couches or beds as available at the location). Teeth were cleaned with cotton rolls but not air-dried prior to examination. Dental assessments included measurement of enamel hypoplasia and opacities, tooth wear, and dental caries and were based on the International Caries Detection and Assessment System (ICDAS). From mid-2015, the presence, presentation, and extent of HSPM were routinely recorded for the buccal, lingual, and occlusal surfaces of the second primary molars per standardized criteria (Ghanim et al. 2015). The presentation of HSPM included demarcated white opacities, demarcated yellow/brown opacities, posteruptive breakdown, atypical restorations, atypical caries, and extractions due to HSPM. However, an HSPM-specific examination was not part of the protocol for approximately 158 children who completed dental examinations prior to mid-2015, who had a broader examination for all developmental defects of enamel (DDEs). Therefore, for these children, already collected data regarding dental caries, opacities, and hypoplasia were reviewed to identify children potentially with HSPM. Children whose second primary molars were all present and had no caries experience (ICDAS caries codes ≤2 and restoration/sealant codes ≤2), no signs of opacities, and no hypoplasia were deemed to be unaffected by HSPM (Fig. 1). All other children ($n = 52$) were reexamined in July 2016 with standardized HSPM criteria.

Data Analysis

Study data were collected and managed with REDCap electronic data capture tools (Harris et al. 2009) and analyzed with STATA 15 (StataCorp; see Appendix). Children with ≥1 HSPM were classified as affected, and those without HSPM were deemed unaffected. Twin pairs where both children were affected by HSPM were classified as concordant, and pairs where only 1 twin was affected were classified as discordant.

To investigate associations between environmental risk factors and presence of HSPM, logistic regression models were fitted with generalized estimating equations (GEEs) to adjust for twin correlation. The outcome variable was binary, presence or absence of HSPM, and the associations were reported as odds ratios (ORs) with 95% CIs.

A comprehensive list of exposure variables was selected from the health and lifestyle data collected during pregnancy, birth, and 18 mo of age, based on biological plausibility and previous evidence of association with MIH or HSPM (Silva et al. 2016; van der Tas et al. 2018). Exposure variables with $P < 0.1$ in the simple regression models were combined in the final multiple regression models to adjust for confounding. Due to the relatively large number of participants missing birth vitamin D data, 2 multiple regression models, with and without the covariate, were considered. As an exploratory study, an inclusive approach was adopted for model building, aiming to identify potential factors rather than exclude factors.

Within-pair analysis to explore the role of categorical nonshared risk factors, such as early life hospitalization and antibiotic use, among twin pairs who were HSPM discordant was planned but not performed, as there were too few twin pairs discordant for both outcome and exposure. To determine whether observed associations with nonshared continuous variables were likely to be causal or due to unmeasured shared or unshared factors, within- and between-pair models were fitted with GEE (Carlin et al. 2005).

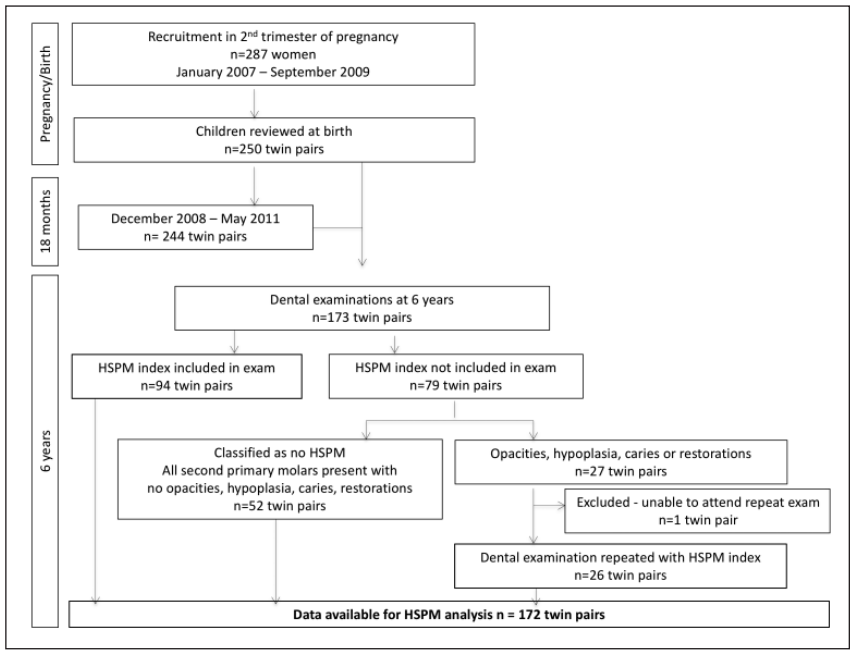

Figure 1. A total of 172 pairs completed dental examinations with HSPM measurement, representing a retention rate of 68.8% from birth. Measurement of HSPM as part of dental examinations commenced July 2015. For the 79 pairs seen prior to inclusion of the HSPM index, 52 were classified as unaffected by HSPM, as they had all 4 second primary molars present, with no evidence of opacities, hypoplasia, caries (ICDAS caries codes 3 to 6), or restorations (ICDAS restorative codes 3 to 8). Of the 29 pairs where at least 1 twin had opacities, hypoplasia, caries, or restorations recorded, 28 were reexamined, and data were excluded from 1 pair that was unable to attend. HSPM, hypomineralized second primary molar; ICDAS, International Caries Detection and Assessment System.

To explore the role of genes and environmental factors, case-wise concordances with 95% CIs were estimated and compared for MZ and DZ twins. To estimate similarities for MZ and DZ twins after adjusting for known risk factors and covariates, a multiple logistic regression model was fitted using a GEE approach (to account for correlation within pairs) to estimate the OR of being affected when an individual's twin is affected. (Ramakrishnan et al. 1992; Betensky et al. 2001).

Results

Participant Demographics

A total of 172 twin pairs (101 DZ and 71 MZ) participated in the dental examinations, representing 68.8% of the original cohort (Fig. 1). The median age was 6.78 y (interquartile range, 6.35 to 7.72), and 184 (53.5%) were female. The mean SEIFA score for the cohort was 1,014.5 (SD, 57.9; Table 1), indicating that the sample had an SES higher than the Australian average and less variation.

HSPM Prevalence

A total of 68 (19.8%) children and 141 (10.2%) teeth had HSPM. Of those with HSPM, 31 (45.6%) had 1; 14 (20.6%) had 2; 10 (14.7%) had 3; and 13 (19.12%) had 4 HSPM. Approximately half of the affected children ($n = 32$, 47.1%) had posteruptive breakdown, caries, atypical restorations, or extractions as a result of HSPM.

HSPM Risk Factors

Dizygosity, age, chorionicity, cord attachment, maternal smoking beyond the first trimester, high SEIFA, in vitro fertilization (IVF), vitamin D levels at birth, and infantile eczema were all associated with HSPM ($P < 0.1$) in the unadjusted regression models (Table 1). Monochorionicity was not included in the multiple regression models due to collinearity with zygosity. When combined in a multiple regression model to adjust for confounding (Table 2), HSPM demonstrated a weak association with dizygosity ($P = 0.099$ and $P = 0.077$) and moderate to strong associations with infantile eczema ($P = 0.055$ and $P = 0.046$), birth vitamin D levels ($P = 0.038$), IVF ($P = 0.005$ and $P = 0.062$), SEIFA score ($P = 0.027$), and maternal smoking beyond the first trimester of pregnancy ($P = 0.064$ and $P = 0.007$).

Within-pair analysis of birthweight, a continuous variable, and HSPM failed to show any association, with a mean within-pair difference between affected and unaffected twins of 28.8 g (95% CI, –92.69 to 150.25; $P = 0.634$) in the 36 pairs discordant for HSPM. The mean within-pair difference in birth vitamin D levels for the 23 twin pairs who were HSPM discordant with measured vitamin D levels was 3.34 nmol/L (95% CI, –4.04 to 10.71; $P = 0.357$). Neither within-pair (OR, 1.87; 95%

Table 1. Simple (Unadjusted) Logistic Regression for Risk Factors for Hypomineralized Second Primary Molars.

Factor	Unadjusted Odds Ratio (95% CI)	*P* Value
Dizygosity ($n = 202$)	2.07 (1.02 to 4.19)	0.043
Age		
Quintile 1, 6.00 to 6.30 y ($n = 70$)	1	Reference
Quintile 2, 6.30 to 6.58 y ($n = 68$)	0.23 (0.07 to 0.72)	0.012
Quintile 3, 6.59 to 7.02 y ($n = 70$)	0.54 (0.19 to 1.58)	0.261
Quintile 4, 7.05 to 7.96 y ($n = 68$)	1.64 (0.72 to 3.74)	0.241
Quintile 5, 7.99 to 9.24 y ($n = 68$)	1.42 (0.59 to 3.42)	0.432
Female ($n = 184$)	0.72 (0.41 to 1.29)	0.272
Maternal school leaving age		
Year 10 ($n = 30$)	1	Reference
Year 11 ($n = 24$)	0.57 (0.12 to 2.81)	0.491
Year 12 ($n = 284$)	1.05 (0.32 to 3.40)	0.937
Socioeconomic indexes for area (per 100 units) ($n = 342$)	1.83 (1.02 to 3.27)	0.042
Maternal		
Body mass index (5 kg/m^2)	1.04 (0.80 to 1.34)	0.789
Stress (per unit)	1.01 (0.98 to 1.05)	0.424
Infection during pregnancy ($n = 204$)	1.14 (0.60 to 2.16)	0.695
Antibiotics use during pregnancy ($n = 66$)	0.78 (0.35 to 1.72)	0.532
Vitamin D at 28 wk (20 nmol/L)	1.17 (0.87 to 1.58)	0.300
Smoking		
During pregnancy ($n = 88$)	1.52 (0.77 to 3.00)	0.225
In first trimester ($n = 86$)	1.58 (0.80 to 3.13)	0.185
In second trimester ($n = 40$)	2.84 (1.28 to 6.30)	0.010
In third trimester ($n = 40$)	3.23 (1.42 to 7.33)	0.005
Alcohol		
Intake ($n = 198$)	1.24 (0.67 to 2.32)	0.495
In third trimester ($n = 76$)	1.64 (0.82 to 3.27)	0.162
Gestational age (wk)	0.98 (0.87 to 1.11)	0.793
Vaginal delivery ($n = 117$)	1.03 (0.56 to 1.89)	0.920
Cord attachment		
Central ($n = 142$)	1	Reference
Peripheral ($n = 120$)	1.33 (0.73 to 2.41)	0.348
Velamentous ($n = 37$)	0.32 (0.10 to 1.05)	0.060
In vitro fertilization ($n = 58$)	2.79 (1.36 to 5.71)	0.005
Chorionicity		
Monochorionic ($n = 96$)	0.39 (0.16 to 0.95)	0.039
Dichorionic ($n = 218$)	1	Reference
Birth weight (100 g)	0.99 (0.94 to 1.05)	0.807
Child vitamin D at birth (20 nmol/L) ($n = 242$)	1.37 (1.00 to 1.87)	0.051
Admission to neonatal intensive care unit / special care nursery ($n = 146$)	1.44 (0.80 to 2.59)	0.220
Breastfeeding		
Ever ($n = 300$)	0.41 (0.13 to 1.24)	0.113
Exclusive to 4 mo ($n = 63$)	0.76 (0.32 to 1.82)	0.541
Hospitalization		
In first 18 mo ($n = 68$)	1.13 (0.58 to 2.23)	0.717
For infection in first 18 mo ($n = 38$)	0.86 (0.31 to 2.39)	0.777
First 18 mo		
Infection ($n = 171$)	1.06 (0.55 to 2.04)	0.856
Nonhospitalized antibiotics ($n = 75$)	1.31 (0.67 to 2.55)	0.429
Asthma ($n = 21$)	0.72 (0.19 to 2.70)	0.626
Eczema ($n = 80$)	2.29 (1.23 to 4.28)	0.009
Food allergy ($n = 81$)	1.08 (0.52 to 2.28)	0.833
Eczema required steroid cream 18 mo to 6 y ($n = 90$)	0.80 (0.38 to 1.68)	0.549
Asthma 18 mo to 6 y ($n = 55$)	0.59 (0.21 to 1.66)	0.320

Moderate to strong evidence ($P < 0.1$) was found for an association with vitamin D levels at birth, eczema before 18 mo of age, in vitro fertilization, dizygosity, age at examination, maternal smoking in the second and/or third trimester of pregnancy, chorionicity, and cord attachment.

CI, 0.59 to 5.88; $P = 0.284$) nor between-pair (OR, 1.42; 95% CI, 0.93 to 2.17; $P = 0.102$) differences in birth vitamin D levels were associated with HSPM, after adjusting for the effect of infantile eczema, maternal smoking, dizygosity, and IVF.

HSPM Concordance

Both twins were affected in 16 twin pairs, and only 1 twin was affected in 36 pairs. The overall concordance for HSPM was

Table 2. Factors Associated with HSPM after Adjusting for Confounding.

Factor	Model 1[a] ($n = 215$)		Model 2[b] ($n = 283$)	
	Adjusted Odds Ratio (95% CI)	*P* Value	Adjusted Odds Ratio (95% CI)	*P* Value
SEIFA (per 100 units)			1.95 (1.07 to 3.53)	0.027
Maternal smoking in second/third trimester	3.64 (0.93 to 14.30)	0.064	3.85 (1.44 to 10.29)	0.007
Eczema in first 18 mo	2.28 (0.98 to 5.27)	0.055	2.05 (1.01 to 4.13)	0.046
Dizygosity	2.49 (0.84 to 7.40)	0.099	1.99 (0.93 to 4.27)	0.077
Birth vitamin D (per 20 nmol)	1.58 (1.03 to 2.43)	0.038		
In vitro fertilization	3.67 (1.48 to 9.12)	0.005	2.37 (0.96 to 5.89)	0.062

HSPM, hypomineralized second primary molar; SEIFA, Socio-economic Indexes for Areas.
[a]Model 1 includes factors found to be associated with HSPM after adjusting for confounding by covariates identified in the unadjusted logistic regression, and it includes birth vitamin D levels as a potential exposure. Pseudo $R^2 = 0.131$.
[b]Model 2 includes factors found to be associated with HSPM after adjusting for confounding by all factors, and it does not include birth vitamin D levels. Pseudo $R^2 = 0.110$.

0.47 (95% CI, 0.32 to 0.62) in the cohort (Fig. 2). The moderate degree of concordance in the cohort suggests that shared factors (either genetic or environmental) are important in the etiology of HSPM.

There was weak evidence of higher unadjusted concordance for MZ twins (0.63; 95% CI, 0.38 to 0.89) than for DZ twins (0.41; 95% CI, 0.24 to 0.58), with a difference of 0.22 (95% CI, –0.09 to 0.53; $P = 0.078$; Fig. 2). This therefore provides only weak evidence for a genetic influence on the risk of HSPM, although the confidence interval for the difference is also consistent with moderate genetic influences.

After adjusting for known risk factors—maternal smoking beyond the first trimester of pregnancy, infantile eczema, dizygosity, IVF, and birth vitamin D—the OR of an individual with an affected twin who had HSPM was 3.97 (95% CI, 1.35 to 11.67; $P = 0.012$). Furthermore, the OR of a DZ twin having an affected twin was 2.49 (95% CI, 0.83 to 7.51; $P = 0.104$), as opposed to 17.69 (95% CI, 1.36 to 230.52; $P = 0.028$) for an MZ twin. However, there was little evidence for a difference between these ($P = 0.172$), consistent with no additive genetic influences on HSPM after adjusting for known risk factors.

Results were robust to the exclusion of outlying and influential observations.

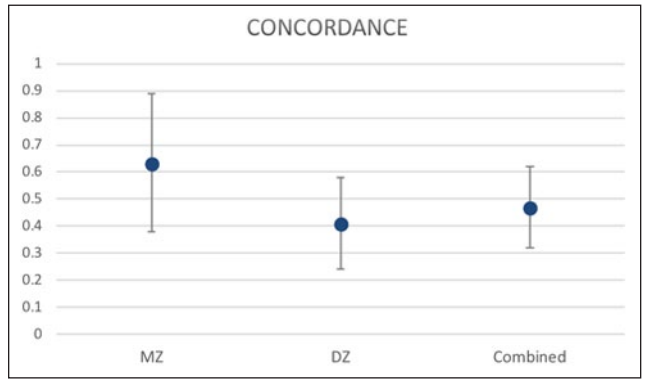

Figure 2. The overall high concordance (0.47; 95% CI, 0.32 to 0.62) in the cohort suggests that shared factors are important. As monozygotic (MZ) twins share 100% of their genes and dizygous (DZ) twins share 50%, a strong genetic influence would lead to a significant difference in concordance between MZ and DZ pairs. Although MZ twin concordance (0.63) is higher than DZ twin concordance (0.41), large confidence intervals provide only weak evidence of a genetic role in etiology.

Discussion

The prevalence of HSPM in this study was 19.8%, higher than the previously reported prevalence of 4% to 14.5% (Elfrink et al. 2008; Ghanim et al. 2013; Mittal and Sharma 2015; Temilola et al. 2015; Negre-Barber et al. 2016; Owen et al. 2018). Developmental defects of enamel (DDE) are considered more prevalent in twins due to the increased frequency of pre- and perinatal complications, possibly accounting for the high prevalence in this cohort (Taji et al. 2011). Although the international prevalence of MIH has been relatively widely explored, prevalence data for HSPM are limited and potentially inaccurate because of inconsistent study methodology and diagnostic criteria (Elfrink et al. 2015). This is an important knowledge gap, given the high associated morbidity.

This study showed that shared environmental factors are more important in HSPM etiology than genetics, although the lower number of MZ twins may have affected the power of the study to detect a higher concordance as compared with DZ twins. The association with dizygosity (a shared factor) is difficult to explain and may be a marker for another exposure that has not been measured, or it may be an incidental finding. Larger twin studies would enable further exploration of this association. With the exception of 1 Indian study that revealed no association between IVF and DDEs in the primary dentition, there have been limited investigations into the impact of IVF on dental health (Kar et al. 2014). Higher rates of perinatal adverse outcomes and congenital abnormalities from pregnancies conceived via IVF have been attributed to parental (i.e., smoking) and technique-related factors, and these may also be important in the etiology of MIH and HSPM (Pinborg et al. 2012). Given the rapidly increasing rates of IVF treatment worldwide, further investigations into possible links between IVF and MIH, HSPM, and dental health are needed (Dyer et al. 2016). Maternal smoking in the second and third trimesters is another potentially important shared environmental exposure. In animal studies, smoke exposure in utero influences

ameloblast function and has also been linked with hypodontia in humans (Dong et al. 2011; Al-Ani et al. 2017). Taken with the findings of the present study, further research is warranted into maternal smoking and its role on HSPM and dental development more broadly. Social advantage has also been linked with MIH, but data are inconsistent and the relationship between MIH/HSPM and SES needs further examination/exploration (Temilola et al. 2015).

Even after adjusting for known risk factors, there was a high degree of similarity within twin pairs in the cohort (as measured by twin OR), suggesting that other shared factors are important. In contrast to a lower-powered twin study of MIH (Teixeira et al. 2018), comparison of concordances for MZ and DZ twins after adjusting for known risk factors revealed no evidence of a strong genetic influence; therefore, other shared environmental factors not measured or underpowered in this study are likely to influence etiology.

Although this study provides strong evidence for an etiologic role of shared factors, nonshared factors were also associated with HSPM. The unexpected association between HSPM and higher levels of vitamin D was not supported by the within-pair and "within- and between-pair" analyses. As within-pair twin analyses control for measured and unmeasured factors, they are superior to general regression models, which can account only for measured factors, although they may be lower powered. Therefore, within-pair analyses can shed further insight on whether an association is more likely to reflect causality or simply shared confounding. This is particularly relevant in this example and suggests that the association between vitamin D and HSPM may be due to confounding by another unmeasured risk factor for HSPM. Nevertheless, a recent prospective study found lower odds of HSPM with lower levels of vitamin D, although these associations were not sustained after adjusting for confounding by known risk factors for HSPM (van der Tas et al. 2018). However, given the biological importance of vitamin D in dental development, the association between HSPM and vitamin D warrants further investigation.

The relationship between eczema and oral health has not been widely studied and never previously associated with HSPM or MIH. The authors of a Singaporean cohort study attributed higher caries risk among children with atopic dermatitis to susceptibility to DDEs but did not measure these directly (Kalhan et al. 2017). Given that infantile eczema occurs after the period when second primary molars would be considered to be susceptible to HSPM, any association with eczema is more likely to reflect common developmental pathways occurring prenatally.

The links with infantile eczema, maternal smoking, and socioeconomic position may suggest an etiology similar to allergy and atopy, mediated through microbiome and immunity (Lewis and Britton 1998). However, no association was found between HSPM and asthma or food allergies in either the mothers during pregnancy or the children. Further research into common developmental pathways between infantile eczema and DDEs and more detailed diagnosis and classification of early childhood allergy and related conditions may provide insight into potential mechanisms underlying the association between HSPM and eczema.

Another notable finding in this study is the lack of evidence for an association between HSPM and factors traditionally considered important. Observational studies have linked HSPM with a range of early-life environmental factors, including maternal alcohol intake during pregnancy, low birth weight, and early childhood illness (Ghanim et al. 2012; Elfrink et al. 2014). Early childhood illness was reported to have the strongest association with MIH (Silva et al. 2016). However, most studies are retrospective and prone to recall bias, and the present study suggests that these may have misrepresented the importance of some of these factors in the etiology of HSPM.

Twin studies have traditionally been used to determine the heritability (or genetic contribution) of conditions. However, although insightful, this is prone to misinterpretation and oversimplification of the complex interactions that underlie conditions such as HSPM. Based on the twin model to explore how known environmental risk factors affect the contribution of genetic and environmental factors, our study has started to explore this complex relationship between nature and nurture.

The current findings must be considered within a number of limitations. Although there is little evidence to suggest that the etiology of HSPM is different between singletons and twins, replication of these findings among singleton cohorts is recommended. A number of factors, such as maternal smoking and childhood illness, though prospectively collected, were based on parental report (medical records were available for pregnancy and birth). Although a high retention rate was maintained, the sample size may have limited the effectiveness of some statistical analyses. The birth vitamin D levels reported in this article may have been influenced by the use of different samples (serum and plasma) and measurement at different time points, despite statistical methods employed to adjust appropriately. As the ideal age for epidemiologic studies of HSPM is 5 y (Elfrink et al. 2015), the slight delay in examination may have led to some underreporting of the condition. Only 1 measure of SES was used, and this was an area level, rather than an individually measured exposure, and so may not accurately reflect individual SES; as such, benefit may have been gained from including other indicators. Finally, the weak evidence for a difference in concordances between MZ and DZ twins may have been due to lack of power.

Conclusion

The prevalence of HSPM in a cohort of 6-y-old Australian twins was 19.8%. With an overall concordance of 0.47, shared factors appear important in the etiology of HSPM. However, due to the lack of a significant difference in concordance between MZ and DZ twin pairs, there was little evidence for a genetic influence, even after adjusting for known risk factors. By applying various twin and other statistical modeling, infantile eczema, vitamin D at birth, IVF, maternal smoking beyond the first trimester of pregnancy, and higher SES were found to be risk factors for HSPM.

Author Contributions

M.J. Silva, contributed to conception, design, data acquisition, analysis, and interpretation, drafted the manuscript; N.M.

Kilpatrick, D.J. Manton, contributed to conception, design, data analysis, and interpretation, critically revised the manuscript; D. Burgner, contributed to data analysis and interpretation, critically revised the manuscript; J.M. Craig, contributed to conception, design, data acquisition, analysis, and interpretation, critically revised the manuscript; P. Leong, contributed to data acquisition, critically revised the manuscript; K.J. Scurrah, contributed to data analysis, critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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