Utilising surface-level data to explore surface, tooth, individual and family influence on the aetiology of hypomineralised second primary molars

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\textbf{ABSTRACT}

\textbf{Objectives:} Hypomineralised second primary molars (HSPM) are common developmental enamel defects. The aims of this study were to use surface-level data to explore the clustering of HSPM at four levels (family, child, tooth, surface).

\textbf{Methods:} This study of 172 twin pairs was nested within the Peri/postnatal Epigenetic Twin Study. HSPM was measured by standardised oral examinations at age 6 years. Multilevel logistic regression models were fitted to assess the correlation structure of surface level data and variation in HSPM. The associations between surface level risk factors and HSPM were then explored using the multilevel logistic regression model using the best fitting correlation structure.

\textbf{Results:} The prevalence of HSPM was 68 (19.8%) children, with a total of 141 (10.3%) teeth and 264 tooth surfaces (6.3%) affected. Multilevel models revealed that a hierarchical structure accounting for correlation at the family, child and tooth level best accounted for the variation in HSPM. The estimated variances from the best fitting model (Model 3) were largest at the family level (12.27, 95% CI 6.68, 22.51) compared with 5.23 at the child level and 1.93 at the tooth level. Application of regression analysis utilising this three-level correlation structure identified tooth/surface level factors in addition to the previously identified familial and individual risk factors for HSPM.

\textbf{Conclusion:} In addition to familial (environmental and genetic) and unique child-level factors, the aetiology of HSPM is likely to be influenced by local tooth-level factors.

\textbf{Clinical Significance:} Clinicians advising patients with HSPM about potential causes should recognise the complex multi-level aetiological influences on the condition.

\textbf{1. Introduction}

Hypomineralised second primary molars (HSPM) are characterised by demarcated opacities of systemic origin, affecting one or more of the second primary molars [1]. HSPM is a risk factor for a similar condition affecting the permanent dentition, molar incisor hypomineralisation (MIH) [2, 3]. Unlike hypoplasia which describes quantitative defects, affected enamel in HSPM and MIH is of normal thickness but qualitatively defective and susceptible to rapid breakdown [4]. HSPM is therefore a risk factor for dental caries [5].

Observational studies have attempted to identify genetic and environmental aetiological factors for HSPM; however, major aetiological
factors remain unknown. Pre- and perinatal events, such as early life illness, may lead to HSPM, but evidence is conflicting [6]. In a previous study, we demonstrated that shared environment may be more important than genetics in the aetiology of HSPM and identified a number of potential aetiologic factors, including maternal smoking in pregnancy and in-vitro fertilization [7].

In contrast to most other systemic perturbations of enamel formation (for example early use of high dose tetracyclines), MIH and HSPM are characterised by inconsistent and unpredictable tooth and surface involvement and severity, between and even within individuals [8]. The clinical presentation varies from a single defect involving less than one third of one surface of one tooth, to extensive involvement of multiple surfaces on multiple teeth. Despite a focus on systemic causes, these patterns suggest that local factors may also be relevant [8]. For example, the detection of foetal isoforms of serum albumin in MIH lesions has recently led to suggestions that the aetiology relates to localised exposure of immature enamel to serum albumin, rather than systemic ameloblast injury [9].

Many epidemiological studies adopt the gold standard for outcome measurement by scoring MIH/HSPM at surface level, rather than at a tooth or individual level. However, this granularity of data is generally lost during analysis, as data are collapsed into binary presence/absence of disease at the individual level [10]. Therefore, risk factor and causal inference analyses may be more insightful if surface level data are utilised. However, such surface level data is not independent, and is likely to be clustered at a number of potential levels including families, individuals, teeth and surfaces, reflecting the pattern of MIH/HSPM at these levels, and the likely underlying aetiology. For example, tooth-level data are not independent and will be clustered according to individuals – with those who have the condition, and therefore the potential systemic risk factors, likely to have multiple teeth affected. Similarly, affected teeth are likely to have multiple surfaces affected whereas unaffected teeth will have none, reflecting another level of clustering. As multiple levels of clustering are possible, relevant hierarchies must be considered and addressed. In addition, utilising twin and family studies provide distinct advantages over studies of unrelated individuals, because clustering within families or twin pairs can be used to evaluate familial risk.

The aims of this observational study of twin children were to explore the clustering of HSPM measured at surface-level with four potential hierarchical levels (family, child, tooth, surface) and discuss implications for understanding the aetiology of MIH and HSPM.

2. Methods

Ethics approval for this study was obtained from the Hospital’s Human Research Ethics Committee (33,174 A) and informed consent was obtained from parents. This observational study conforms to the STROBE guidelines.

This study was part of a larger prospective longitudinal birth cohort of 250 twin children, the Peri/postnatal Epigenetic Twins Study (PETS). A detailed description of the cohort has been reported previously [11]. Women, pregnant with twins, were recruited mid-gestation. The socioeconomic status (SES) of participants at birth was obtained by linking to the Bureau of Statistics based on census data, via residential postcodes [12]. Socio-economic Indexes for Areas (SEIFA) developed by the Australian Bureau of Statistics based on census data, via residential postcodes [12].

In order to demonstrate the utility of the mixed-effects logistic regression model with the best fitting correlation structure, a risk factor analysis was then undertaken to evaluate the association between HSPM and the buccal, lingual and occlusal surfaces of the second primary molars, as per standardised and validated criteria [10]. The presentation of HSPM included demarcated white opacities, demarcated yellow/brown opacities, post-eruptive breakdown, atypical restorations, atypical caries and extractions due to HSPM. Although surfaces cannot be viewed when teeth have been either extracted or restored with pre-formed metal crowns due to HSPM, the judgement criteria require all three surfaces in such cases to be marked as affected. The extent of surface involvement was classified as less than one third, one to two thirds or more than two thirds of the surface. As MIH-specific criteria were not part of the protocol for approximately 158 children who completed dental examinations prior to mid-2015, a subset of children was re-examined in July 2016.

2.1. Data analysis

Study data were collected and managed using REDCap electronic data capture tools [13] and analysed using Stata 15 (StataCorp. 2017. Stata Statistical Software: Release 15. TX, USA).

2.1.1. Part 1: best fitting multilevel correlation structure

The outcome variable was binary, presence or absence of HSPM, where surfaces with HSPM affected were coded as 1, and those without HSPM affected were coded as 0. The dataset comprised measurements of HSPM for each of three surfaces $j$ for each of four teeth $k$ nested in each of twin children $i$.

Generalised linear mixed models (GLMM), specifically multilevel logistic regression models, which allow both fixed and random effects, were fitted to investigate the multilevel structure of the data.

To determine the most appropriate correlation structure for the HSPM data, four different GLMMs were fitted

1. single-level model including a random intercept at the child level.
2. two-level model with
   a) random intercepts at the child and tooth level.
   b) random intercepts at the child and surface levels.
3. three-level model with random intercepts at the family, child and tooth levels.

Models 2a and 2b were both two-level regression models but differed according to whether the correlation was at the tooth or surface level (in addition to the child level). This was to test whether the correlation between the same surfaces of different teeth was stronger than the correlation between different surfaces within the same tooth. Model 3 evaluated whether adjusting for the correlation due to familial factors improved the fit of the model, as the cohort comprised pairs of twins. Intraclass correlations (ICC) and their 95% confidence intervals were calculated for each of the three levels (tooth, child, and family) using the estimated components of variance for each level, from the best-fitting models.

Akaike’s Information Criterion (AIC) and the Bayesian information Criterion (BIC) were calculated to compare how well the models fitted the observed data. Most models were fitted using mean-variance adaptive Gauss-Hermite quadrature as implemented in the melogit command, but some models were also fitted using Bayesian Markov chain Monte Carlo (MCMC) estimation to assess the extent of potential biases from the likelihood-based approach.

As the presence of HSPM on surfaces of teeth that were either extracted or restored with pre-formed metal crowns was uncertain, the analyses were also repeated excluding data from 9 children with extractions or preformed metal crowns.

2.1.2. Part 2: application of multi-level modelling to explore risk factors

In order to demonstrate the utility of the mixed-effects logistic regression model with the best fitting correlation structure, a risk factor analysis was then undertaken to evaluate the association between...
potential exposures and HSPM. As a proxy of tooth/surface level risk factors, the tooth surface (e.g., upper tooth, buccal surface) was included as a covariate in the multi-level regression. The associations were reported as odds ratios (OR) with 95% confidence intervals (CI) and p-values. As individual (birth weight, early life illness) and family-level factors (maternal smoking, IVF) factors have already been investigated in our previous study, a multiple regression model including these factors is provided in the Supplementary Material.

3. Results

Data from a total of 172 mothers with 344 children (and therefore 1376 second primary molars and a total of 4128 surfaces) were available for analysis. The median age of participants was 6.78 years (IQR 6.35, 7.72) and 184 (53.5%) were female.

The prevalence of HSPM was 68 (19.8%) children, with a total of 141 (10.3% of 1376) teeth and 264 tooth surfaces (6.3% of 4128) affected. Of the 68 affected children, more than half had more than one tooth and more than one surface with HSPM (Table 1). The prevalence of HSPM was slightly lower (15.2%) in children examined at home compared with those examined onsite at the research institute (20.1%), but the number of children with opacities only was similar (10.8% of children examined at home and 9.1% of children examined onsite).

3.1. Teeth and surfaces affected

Of the 141 teeth affected by HSPM, more than half had multi-surface involvement (Table 1). Of the 68 children with HSPM, teeth 55, 65, 75 and 85 were affected in 36, 31, 41 and 33 children respectively (Table 3). The maxillary teeth had higher rates of HSPM on palatal surfaces, but the buccal surfaces were more commonly affected on mandibular teeth (Table 2).


The estimated variances for each of the four unadjusted models with the associated log-likelihood, AIC and BIC for each of these models and the null model are presented in Table 4 (additional modelling provided in Online Resource). The best fit was found for Model 3, which allowed correlation at the family, child and tooth level (AIC=1148.23).

With regard to the alternative correlation structures, it is evident that even simply accounting for child-level correlation in Model 1 (AIC=1186.75) made a significant improvement over the null model which assumed independence (AIC=1910.45) and therefore did not account for clustering within the data. With regards to the two-level models, Model 2a, which allowed correlation at both child and tooth levels fitted better (AIC of 1167.90) than Model 2b (AIC=1186.75), which allowed for correlation at child and surface levels. This suggests that there was no correlation between the equivalent surfaces of different teeth.

3.3. Multi-level modelling – variance components

The estimated variances from the best fitting model (Model 3) were largest at the family level (12.27, 95% CI 6.68, 22.51) compared with 5.23 at the child level and 1.93 at the tooth level. Therefore, in addition to a major contribution from familial and child-level factors, there are also smaller additional influences at tooth-level. From this model, the ICC at the family level was 0.54 (95% CI 0.38, 0.70) while the ICC for the child level (within a family) was 0.77 (95% CI 0.68, 0.84) and the ICC for tooth (within a child within a family) was 0.86 (95% CI 0.79, 0.90).

3.4. Risk factor analysis

Results from the best-fitting multivariable GLMM (Model 3, correlation at tooth, child and family levels) including a surface level covariate are reported in Table 4. There was strong evidence that buccal surfaces of lower teeth are at increased risk of HSPM. Previously reported individual and family-level risk factors (IVF, high socio-economic status, maternal smoking beyond the first trimester of pregnancy and eczema in first 18 months) were also associated with risk of HSPM when this multi-level model was fitted to surface data. However, estimates from the regression model including these additional factors were highly inflated, with large ORs and wide confidence intervals, probably reflecting a lack of information and power in the current data set. These secondary results were therefore considered unreliable (Supplementary Material). Sensitivity analyses suggested that the extremely high estimated OR for IVF may also be due to several children severely affected by HSPM (9 children with extractions or preformed metal crowns, of whom 5 had more than 9 affected surfaces). When models were refitted excluding data for children with extractions or stainless steel crowns, results were qualitatively similar although less extreme (Supplementary Material). Results were also similar when Bayesian MCMC estimation was used (data not shown).

4. Discussion

To our knowledge, this is the first study to apply multi-level models and utilise surface-level data to explore the aetiology of HSPM. The correlation structure demonstrates that in addition to a major contribution from familial and child-level factors, there are also smaller additional influences at tooth-level.

At almost 20%, the prevalence of HSPM, is higher than previously reported in singleton studies of HSPM [14]. Twin births are more likely to be associated with pre- and perinatal complications, increasing the risk of developmental defects of enamel (DDE) such as HSPM [15]. Buccal surfaces have been reported to be more commonly affected by hypomineralisation in HSPM and MIH. However, in our study, only the buccal surfaces of mandibular teeth demonstrated higher risk of HSPM. This apparent discrepancy between our findings and other studies may be because others frequently do not report the surfaces affected separately for the maxillary and mandibular arches [16, 17]. Differences in visibility of buccal mandibular surfaces, particularly in a non-dental specific setting may also account for the reported higher rates of defects on these teeth.

In demonstrating the utility of surface-level analysis of HSPM risk factors, this analysis was limited as it was only possible to consider a single surface-level covariate [7]. However, utilising surface level data that accounted for the correlation structure of the data and inclusion of

<p>| Table 1. The number/frequency of surfaces affected by HSPM at child and tooth level. |
|---------------------------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Number of surfaces affected</th>
<th>Number of children (n = 344)</th>
<th>Number of teeth (n = 1376)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>276 (80.2)</td>
<td>1235 (89.7)</td>
</tr>
<tr>
<td>1</td>
<td>22 (6.4)</td>
<td>64 (4.7)</td>
</tr>
<tr>
<td>2</td>
<td>13 (3.8)</td>
<td>31 (2.3)</td>
</tr>
<tr>
<td>3</td>
<td>9 (2.6)</td>
<td>46 (3.4)</td>
</tr>
<tr>
<td>4 – 9</td>
<td>17 (4.9)</td>
<td>N/A</td>
</tr>
<tr>
<td>10+</td>
<td>7 (2.1)</td>
<td>N/A</td>
</tr>
</tbody>
</table>

<p>| Table 2. Surfaces affected by HSPM. |
|---------------------------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Tooth</th>
<th>Total number of teeth affected in cohort (%)</th>
<th>Buccal surfaces affected (% of affected teeth)</th>
<th>Occlusal surfaces affected (% of affected teeth)</th>
<th>Palatal surfaces Affected (% of affected teeth)</th>
</tr>
</thead>
<tbody>
<tr>
<td>55</td>
<td>36 (10.5)</td>
<td>14 (38.9)</td>
<td>22 (61.1)</td>
<td>29 (80.6)</td>
</tr>
<tr>
<td>65</td>
<td>31 (9.0)</td>
<td>13 (41.9)</td>
<td>22 (71.0)</td>
<td>22 (71.0)</td>
</tr>
<tr>
<td>75</td>
<td>41 (11.9)</td>
<td>34 (82.9)</td>
<td>23 (56.1)</td>
<td>16 (39.0)</td>
</tr>
<tr>
<td>85</td>
<td>33 (9.6)</td>
<td>29 (87.9)</td>
<td>19 (57.6)</td>
<td>11 (33.3)</td>
</tr>
<tr>
<td>Total</td>
<td>141 (10.3)</td>
<td>90</td>
<td>86</td>
<td>78</td>
</tr>
</tbody>
</table>
aetiological factors may be appropriate given the definition of MIH and severity of HSPM can vary markedly at the tooth and surface level even measurement, making comparisons between studies difficult [19]. This study demonstrating tooth-level correlation, inclusion of such patterns, arising from the buccal aspect, and therefore increasing susceptibility on buccal surfaces [8].

Given the variable presentation of HSPM and MIH and evidence from this study demonstrating tooth-level correlation, inclusion of such potential local factors (e.g., epigenetics and presence of other anomalies in the quadrant) in future studies might enable more meaningful insights.

Our study highlights the importance of recording enamel hypomineralisation at surface and tooth levels. Early criticisms of observational studies of MIH noted the lack of standardised outcome measurement, making comparisons between studies difficult [19]. However, the establishment of EAPD criteria led to improvement in consistency of MIH and HSPM measurement [5]. The development of validated indices that enable accurate measurement of MIH and HSPM also supports researchers to undertake robust observational studies of MIH and HSPM [10, 20, 21]. However, the scoring of teeth that were either restored with preformed metal crowns or extracted due to MIH/HSPM remains a potential source of inaccuracy because all surfaces are marked as affected without the surface being visualised. Consequently, it is possible and quite likely that in many cases with either crowns or extraction(s), not all tooth surfaces were affected.

Overcoming this issue is difficult and would require examination of the teeth soon after eruption but prior to treatment, which given the variable nature of tooth eruption, is difficult to predict for population-based research. In addition, the classification system used to quantify HSPM, though validated and widely used, does not include measurement of tooth hypersensitivity, a metric that has been proposed for inclusion in measurement of disease severity [22, 23]. However, the use of precise and highly specialised indices creates challenges in relation to the cost and time needed to complete these dental examinations. This has made inclusion in prospective cohort studies difficult, compromising other important aspects of observational studies of aetiology, including the need for accurate, prospectively collected exposure data. These limitations may be overcome in future studies by utilising registry or clinical data from health services, with the caveat that accurate clinical records are maintained, or use of imaging such as digital photography or intra-oral scanning.

We acknowledge a number of limitations. Despite inclusion of surface level data, it was not possible to include lesion descriptions or extent of surface involvement. There is strong evidence to suggest that surface extent and worsening severity align with the number of affected teeth and therefore maybe of limited additional benefit [24]. Nevertheless, the variable presentation of MIH and HSPM may span all three aspects (number of surfaces, extent and presentation) and adds further complexity to understanding aetiology. As a single centre study, the findings should be generalised with caution particularly given that as a longitudinal twin cohort, the study sample may not be an accurate representation of the community. Geographic and genetic variation have both been reported in MIH and HSPM and therefore further studies in ethnically and geographically diverse settings are recommended [25]. Finally, although the study represents a relatively large twin cohort study with excellent retention, the relatively small sample size is likely to limit some interpretation with estimates greatly influenced by a small group of severely affected children.

5. Conclusion

Moderate correlation at family-level suggests that shared familial factors are important determinants of HSPM. Additional correlation at child- and tooth-level also suggest the presence of unique child and oral factors that warrant further investigation. Given the variable presentation of MIH and HSPM in individuals, utilising surface level data may provide valuable insights into the aetiology of MIH and HSPM. Such analyses are likely to be more meaningful with the inclusion of tooth-level factors.

Author contribution

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Mihiri Silva, Nicky Kilpatrick, Jeff Craig, Ying Zheng, Katrina Scurrah, Sophie Zaloumis. The first draft of the manuscript was written by Mihiri Silva.

Table 3.
Estimated variation from the four mixed effects logistic regression models including different random intercept terms.

<table>
<thead>
<tr>
<th>Correlation</th>
<th>Model 0: No correlation</th>
<th>Model 1: Single-level correlation</th>
<th>Model 2: Two-level correlation</th>
<th>Model 3: Three-level correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Child level</td>
<td>Child and tooth levels (Model 2a)</td>
<td>Child and surface levels (Model 2b)</td>
<td>Family, child, tooth level</td>
</tr>
<tr>
<td></td>
<td>Estimate 95% CI</td>
<td>Estimate 95% CI</td>
<td>Estimate 95% CI</td>
<td>Estimate 95% CI</td>
</tr>
<tr>
<td>Family</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Children</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Teeth</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surfaces</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Log Likelihood</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AIC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BIC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4.
Results from final logistic regression model examining the association between risk factors and HSPM using surface-level data.

<table>
<thead>
<tr>
<th>Fixed Effects</th>
<th>Model 3a including all participants (n = 3840)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR 95% CI</td>
</tr>
<tr>
<td>Surface</td>
<td></td>
</tr>
<tr>
<td>Occlusal surface, lower tooth</td>
<td>1.0</td>
</tr>
<tr>
<td>Buccal surface, lower tooth</td>
<td>3.28</td>
</tr>
<tr>
<td>Lingual surface, lower tooth</td>
<td>0.29</td>
</tr>
<tr>
<td>Occlusal surface, upper tooth</td>
<td>1.11</td>
</tr>
<tr>
<td>Buccal surface, upper tooth</td>
<td>0.31</td>
</tr>
<tr>
<td>Lingual surface, upper tooth</td>
<td>1.68</td>
</tr>
<tr>
<td>Random Effect Variances (logit scale)</td>
<td></td>
</tr>
<tr>
<td>Mother</td>
<td>16.01</td>
</tr>
<tr>
<td>Child</td>
<td>6.90</td>
</tr>
<tr>
<td>Tooth</td>
<td>3.08</td>
</tr>
<tr>
<td>Log likelihood</td>
<td>–540.01</td>
</tr>
<tr>
<td>AIC</td>
<td>1098.01</td>
</tr>
<tr>
<td>BIC</td>
<td>1154.94</td>
</tr>
</tbody>
</table>

surface-location co-variates enabled inclusion of a much broader range of outcome data and offers considerable potential for future studies. All previous observational studies of MIH and HSPM have investigated aetiology utilising individual-level data despite recognition that the severity of HSPM can vary markedly at the tooth and surface level even within the same individual [7, 18]. Although the inclusion of child-level aetiological factors may be appropriate given the definition of MIH and HSPM, which specifically recognises the cause to be systemic, our findings suggest that local factors are also likely to be contributory and therefore require investigation. Investigators have suggested that signalling that drives tooth formation may follow specific geographic patterns, arising from the buccal aspect, and therefore increasing susceptibility on buccal surfaces [8].
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Ethics and informed consent

Ethics approval for this study was obtained from the Royal Children’s Hospital Human Research Ethics Committee (33174 A) and informed consent was obtained from parents.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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