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The effects of modifiable maternal pregnancy exposures on offspring molar-incisor hypomineralisation: A negative control study

Qui-Yi Lim,[1] Kurt Taylor[2] and Tom Dudding[1]

[1]*Bristol Dental School, UK;* [2]*Bristol Medical School, UK*

Objectives: Explore associations between modifiable maternal pregnancy exposures: pre-pregnancy body mass index (BMI), pregnancy smoking and alcohol consumption with offspring molar-incisor hypomineralisation (MIH) and use negative control analyses to explore for the presence of confounding. ***Method***: Using data from a prospective UK birth cohort, Avon Longitudinal Study of Parents and Children, we performed logistic regression to explore confounder adjusted associations between maternal pre-pregnancy BMI and smoking and alcohol consumption during pregnancy with MIH. We compared these with negative control exposure (paternal BMI, smoking and alcohol) and outcome (offspring dental trauma) analyses. ***Results***: 5,536 mother/offspring pairs were included (297 (5.4%) MIH cases). We found a weak, positive association between maternal mean BMI and offspring MIH (Odds Ratio (OR) per 1-kg/m2 difference in BMI: 1.04, 95% confidence interval (CI): 1.00, 1.08). Results of subsequent analyses suggested this effect was non-linear and being driven by women in the highest BMI quintile (OR for women in the highest BMI quintile versus the lowest: 1.61 95%CI: 1.02, 2.60). Negative control analyses showed no evidence of an association between paternal BMI and offspring MIH (OR: 0.94, 95%CI: 0.89,1.00) and maternal BMI and offspring dental trauma (OR: 0.99, 95%CI: 0.96, 1.02). There was no clear evidence of an association for maternal smoking (OR: 0.76, 95%CI: 0.46,1.22) or alcohol consumption (OR: 0.79, 95%CI: 0.56, 1.21) with offspring MIH with results imprecisely estimated. ***Conclusion***: We found a possible intrauterine effect for high maternal pre-pregnancy BMI on offspring MIH, but no robust evidence of an intrauterine effect for maternal pregnancy smoking or alcohol consumption. A key limitation includes possible misclassification of MIH. Replication of these results is warranted.

Keywords*: Body mass index, Smoking, Alcohol, ALSPAC, Molar-incisor hypomineralisation, Negative control*

Introduction

Molar-incisor hypomineralisation (MIH) is a developmental, qualitative enamel defect caused by reduced mineralisation and inorganic enamel components. It primarily affects one or more first permanent molars (FPMs), and frequently involves incisors (Weerheijm, 2003). MIH is often diagnosed around 6-7 years of age when the FPMs and central incisors erupt into the oral cavity. Due to the poor quality enamel, affected teeth present as aesthetic concerns with discoloured creamy-white or yellow-brown demarcations. These teeth also carry pathological concerns as they are often hypersensitive, susceptible to post-eruptive breakdown (Bullio Fragelli *et al*., 2015) and rapid caries progression (Americano *et al*., 2017). Consequently, MIH-affected teeth often have poor prognosis and are frequently extracted before adulthood.

The prevalence of MIH varies between 2.4 and 40.2%, with a global mean of 13.1% (Schwendicke *et al*., 2018). The literature remains inconclusive around its aetiology; however, it is generally accepted that causes are likely to be multifactorial. Many studies propose a genetic predisposition (Jeremias *et al*., 2016). Although, the clinical presentation of these localised, asymmetrical lesions indicates a further cause of systemic or environmental origin that disrupts enamel formation. The development of FPMs begins *in utero* and continues 2-3 years after birth (Schuurs, 2012; Welbury *et al*., 2018). With this developmental window, a range of prenatal, perinatal, and post-natal factors have been investigated. These include maternal illness and co-morbidities, premature birth, childhood antibiotic exposure, and many more. However, there is little standardisation of these variables, and weak evidence to support any associations with offspring MIH (Fatturi *et al*., 2019; Silva *et al*., 2016).

Despite FPMs developing *in utero* there is little research on maternal exposures during pregnancy. To our knowledge, there no studies have investigated the effect of maternal body mass index (BMI) on MIH. Maternal pre-pregnancy obesity is known to affect foetal development (Leddy *et al*., 2008; Maffeis & Morandi, 2017). FPMs develop *in utero*, therefore, it is plausible that changes in BMI may influence dental development. Effects of pregnancy smoking and alcohol consumption have been well established with birth defects associated with craniofacial and dental abnormalities, such as oral facial clefts for smoking (Little *et al*., 2004), and foetal alcohol syndrome for alcohol (Sant'Anna & Tosello, 2006). Effects of pregnancy smoking and alcohol on MIH have also been investigated. However, recent systematic reviews on these risk factors found no significant evidence of an association (Fatturi *et al*., 2019; Silva *et al*., 2016).

Correspondence to: Qui-Yi Lim. Email: ql16941@bristol.ac.uk

Previous studies have been limited due to a lack of detail or consistency in the maternal exposures and MIH outcomes investigated (Silva *et al*., 2016), which makes comparisons between studies difficult. Many studies have also failed to adjust for confounding, had limited statistical power and predominantly been retrospective, making them prone to common epidemiological biases, such as recall bias. Therefore, we cannot determine whether any apparent relationship reflects the magnitude of the causal effect, or if these results are biased by residual confounding, systematic reporting bias or measurement bias.

Negative control analysis may provide an alternative approach to overcome the limitations of previous studies (Figure 1). This method is used to detect potential confounding after adjusting for measured confounders (Lipsitch *et al*., 2010). Previous studies have used parental negative exposure controls and offspring negative outcome controls as a proxy to explore intrauterine effects of maternal exposures and offspring outcomes (Brand *et al*., 2019; Taylor *et al*., 2021). The idea of negative controls is to substitute a condition with an exposure (Figure 1A) or outcome (Figure 1B) variable that (i) shares similar measured and unmeasured confounding structures as the 'real study' (the original association), and (ii) does not have a plausible biological link with the association of interest (Lipsitch *et al.*, 2010, Gage *et al*., 2016). Under these two assumptions, it can be assumed that the 'real study' and negative control experiment are perfectly comparable, and we can therefore expect results of the negative controls to produce a weaker or no association if there were to be a true causal effect (Lipsitch *et al*., 2010). For example, Figure 1A shows how a negative control exposure, paternal BMI/ smoking/ alcohol can be used as a proxy to investigate effects of intrauterine exposures on MIH birth outcomes. The paternal exposure has the same incoming arrows as the maternal exposure of interest, but has no arrow to the offspring MIH outcome. Therefore, any association observed between the paternal exposure and MIH outcome of interest will be due to confounding in the model. Figure 1B shows how a negative control outcome, offspring dental trauma can similarly be used. Offspring dental trauma has the same incoming arrows as the MIH outcome of interest but has no arrow from the maternal exposure of interest. Therefore, any association observed between the maternal exposure and offspring dental trauma will be due to confounding.

In summary, the exact causes of MIH are unclear and intrauterine factors via maternal pregnancy exposures could be relevant. There is a need for more prospective research using study designs that can assess the presence of confounding. Identifying modifiable risk factors for MIH is important for developing preventive interventions to reduce disease burden.

The aims of this study were to:

1. Explore associations between maternal pre-pregnancy BMI, pregnancy smoking and alcohol consumption with offspring MIH.
2. Use negative control exposure and outcome analyses to explore for the presence of residual confounding.

Methods

We used data from Avon Longitudinal Study of Parents and Children (ALSPAC)**,** an ongoing, multigenerational, prospective birth cohort study including 14,541 pregnant women residing in Avon, UK with expected delivery dates between 1st April 1991 and 31st December 1992. Two further rounds of recruitment totalled 15,454 pregnant women and 14,901 offspring alive at 1 year of age were enrolled in the study (Figure 2) (Boyd *et al*., 2013; Fraser *et al*., 2013)**.** Participants have been regularly followed up through questionnaires and clinic visits at the time of pregnancy in the mothers and fathers, and subsequent offspring from early life through to adulthood. The study website contains details of all the data that is available through a fully searchable data dictionary and variable search tool (http://www.bristol.ac.uk/alspac/researchers/our-data/). Ethical approval for the study was obtained from the ALSPAC Ethics and Law Committee and Local Research Ethics Committees.

At age 7 and 10, participants completed self-report questionnaires (available at http://www.bristol.ac.uk/alspac/researchers/our-data/questionnaires/child-completed-questionnaires/) on dental health, with assistance from their parents and use of a mirror. A subset of participants who had: (i) completed the questionnaire and (ii) had at least one first permanent molar tooth erupted by age 7 were included in this study, totalling 5,536 mother-offspring pairs (Figure 2).

Maternal BMI was calculated using self-reported pre-pregnancy weight and height using data from the questionnaire completed around 12-weeks' gestation. This correlated with the clinically measured pre-pregnancy weight and height. For analyses, BMI was used as a continuous variable. Maternal pregnancy smoking and alcohol consumption were assessed using questionnaires at 18- and 32-weeks' gestation. Mothers reported the number of times smoked per day and how often they consumed alcoholic drinks, measured in glasses per week for each trimester. For smoking, data from each trimester was categorised yes/no, then used to generate one variable of "any pregnancy smoking" (yes/no). Alcohol consumption data, available for the first and third trimester, were used to generate one binary variable, "any pregnancy alcohol consumption" (yes/no). The development of the FPMs begins 3.5-4 months *in utero* and continues after birth (Schuurs, 2012; Welbury *et al*., 2018). Therefore, it is plausible that the intrauterine environment throughout the entire pregnancy could influence offspring MIH.

We defined MIH by using questions related to the child's "6-year molars" from the age 7 and 10 self-report questionnaires. In the age 7 questionnaire, participants were asked to specifically check if each FPM had come through and identify if and any of these had come through brown (Figure S1) (available at http://www.bristol.ac.uk/media-library/sites/alspac/migrated/documents/ques-c07-my-teeth.pdf). Then MIH was defined as "any child who had at least one FPM that came through looking brown" (yes/no).

We used paternal BMI, smoking and alcohol consumption during pregnancy as negative control exposures (Figure 1A). All data used was self-reported by the partners at 18-weeks' gestation (available at http://www.bristol.ac.uk/media-library/sites/alspac/migrated/documents/ques-p02-partners-questionnaire.pdf). Paternal BMI was calculated using self-reported weight and height and used

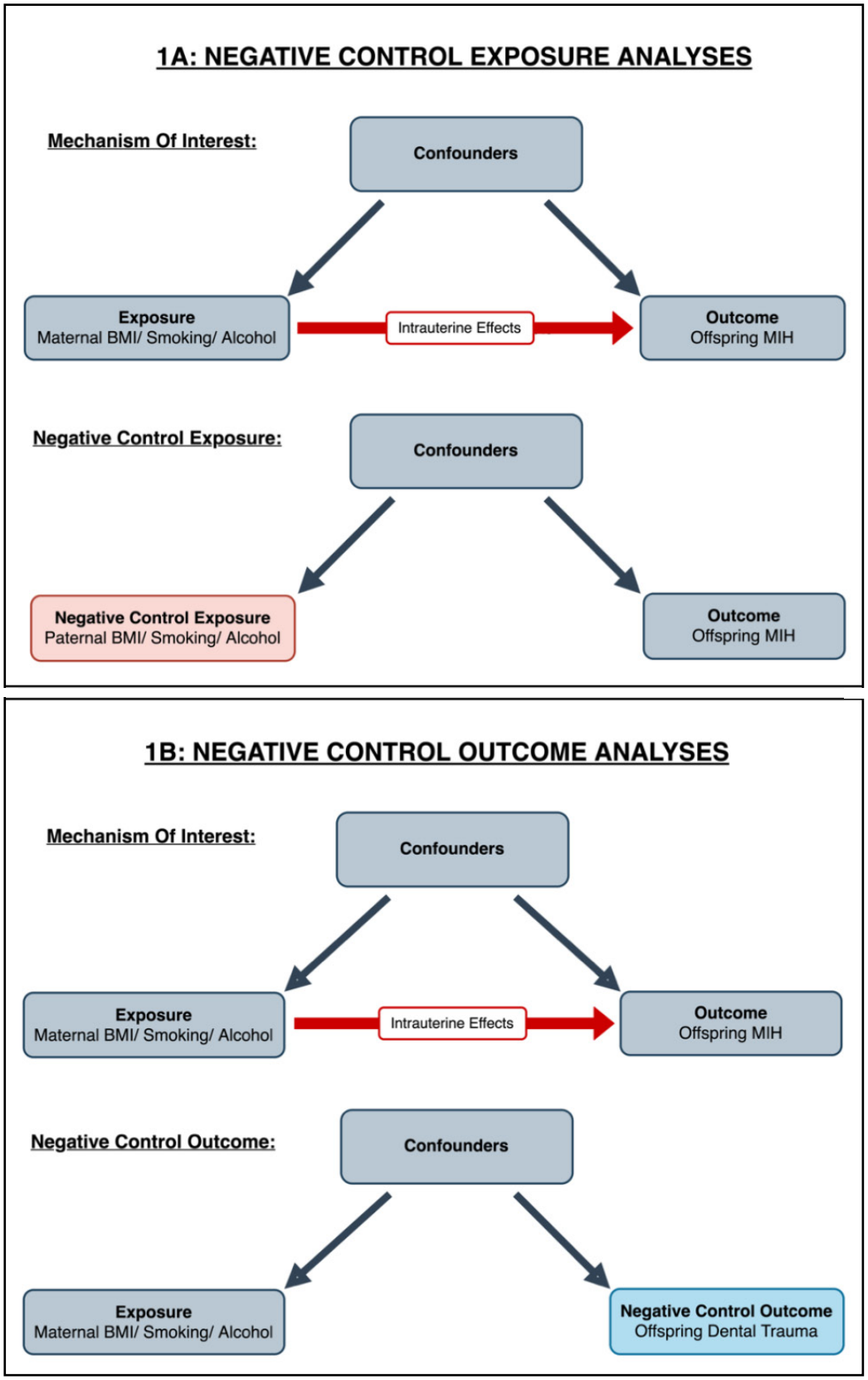

Figure 1. *Negative control experiments.*
1A shows how a negative control exposure, paternal BMI/ smoking/ alcohol can be used to investigate effects of intrauterine exposures on MIH birth outcomes. The paternal exposure has the same incoming arrows as the maternal exposure of interest, but has no arrow to the offspring MIH outcome – this means that any association observed between the paternal exposure and MIH outcome of interest will be due to the confounding variables in the model. 1B shows how a negative control outcome, offspring dental trauma can be used to investigate effects of intrauterine exposures on MIH birth outcomes. Offspring dental trauma has the same incoming arrows as the MIH outcome of interest, but has no arrow from the maternal exposure of interest – this means that any association observed between the maternal exposure and offspring dental trauma will be due to the confounding variables in the model. Each exposure variable (maternal and paternal BMI, smoking and alcohol consumption) and outcome variable (offspring MIH and dental trauma) will be looked at separately.

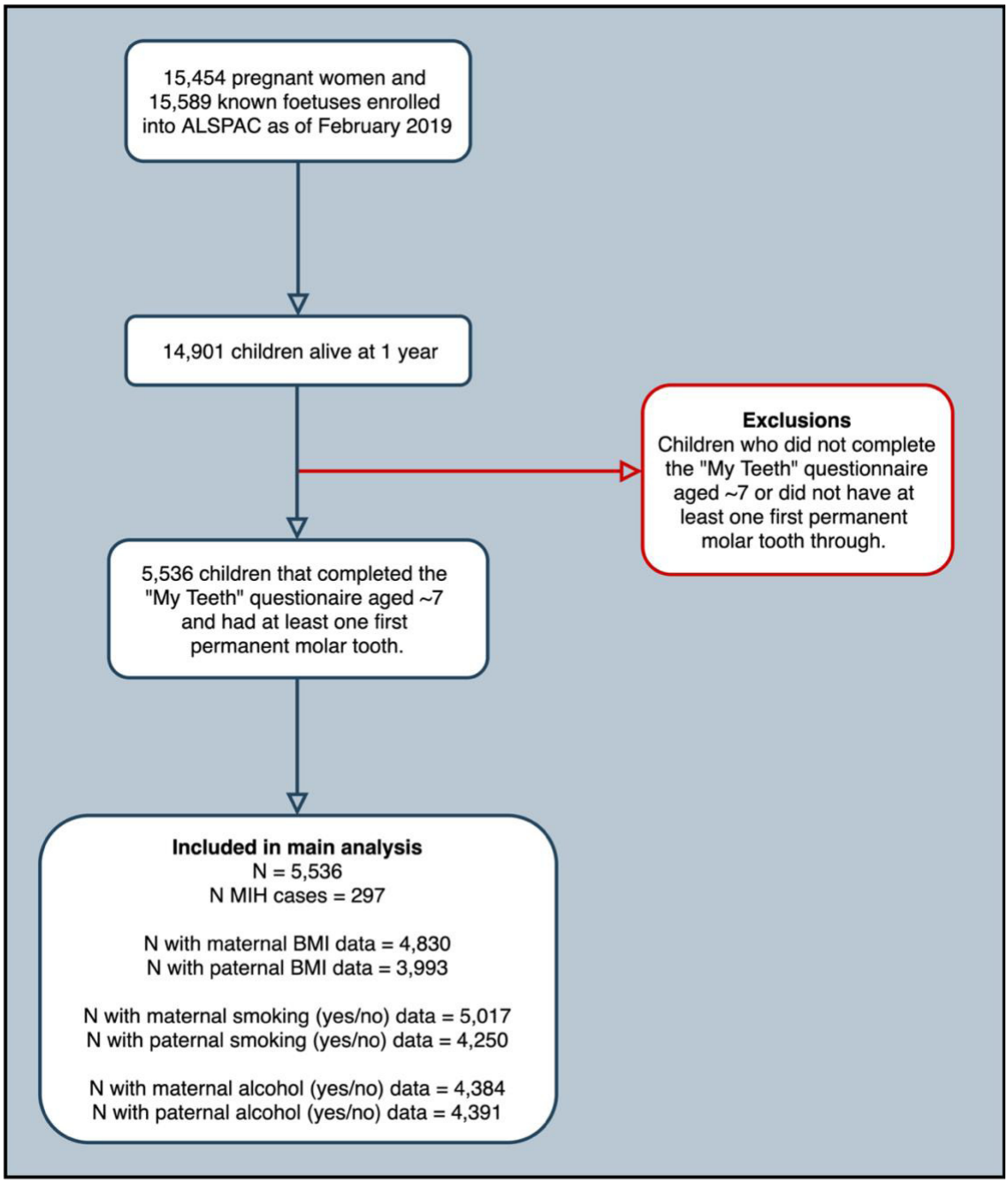

Figure 2. *Participant selection in the ALSPAC cohort.*

as a continuous variable. Paternal smoking habits were measured as the number of times smoked at the start of pregnancy, and defined as a binary variable, "any partner smoking" (yes/no). Paternal alcohol consumption was measured using questions asking how often they consumed alcoholic drinks in the past 3 months, measured in glasses per week, and defined as a binary variable "any alcohol consumption" (yes/no).

We used offspring dental trauma as a negative control outcome (Figure 1B). Questions related to "accidents to your teeth" from the questionnaire answered at age 10 were used (Figure S2B, available in Lim *et al*., 2022). Dental trauma was defined as any child whose teeth became "loose", "chipped" or "knocked out" when they banged their top adult teeth. This negative control outcome was binary, "any dental trauma" (yes/no).

Analyses were adjusted for confounders previously shown to be a plausible influence on maternal BMI, smoking and alcohol exposure and the offspring MIH outcome. The confounders adjusted for in the maternal and paternal analyses include age, education, parity (maternal), smoking (for BMI and alcohol analyses) and alcohol consumption (for BMI and smoking analyses). BMI, smoking, and alcohol were defined as above. Maternal and paternal age were kept as continuous variables. Parity was measured using data from the mother's questionnaire, answered at 18-weeks' gestation. We used questions regarding the mother's previous pregnancies, which asked how many times the mother had previously been pregnant. This was used to define parity as nulliparous and multiparous. Educational attainment was used as a proxy measure of parents' socioeconomic status. We used

questions concerning educational qualifications for both the mothers, and their partners from the mother's questionnaire completed around 32-weeks' gestation. Educational attainment was classified into three categories, low (none/ secondary education or equivalent) medium (vocational/ O/ A level or equivalent) and high (university degree).

Analyses were conducted using R (Version 4.0.3). Analysis steps were discussed and agreed *a priori* and documented (Lim *et al*., 2021). Each exposure variable (maternal and paternal BMI, smoking and alcohol consumption) and outcome variable (offspring MIH and dental trauma) was considered separately. We used logistic regression with maximal numbers (numbers included in each model are likely to vary due to missing data). All analyses were run unadjusted (model 1), confounder adjusted (model 2) and confounder adjusted with the addition of the other parent's exposure (BMI/ smoking/ alcohol consumption) (model 3). Model 3 produces a maternal association that adjusts for maternal confounders as well as the paternal exposure, and similarly a paternal association adjusting for paternal confounders and the maternal exposure, referred to as 'other parent adjusted'. The reason for mutual parent adjustment is that parental BMI, smoking and alcohol consumption may associate with one another through assortative mating, shared social and environmental background, and modelling for each other's behaviours (Sharp & Lawlor, 2019).

For the negative control exposure analyses, all three models were performed, then maternal and paternal exposures were directly compared with MIH outcomes by observing point estimates and 95% Confidence Intervals (CI)s. In the negative control outcome analyses, the first two models of analyses were performed, then maternal exposures were directly compared with MIH and dental trauma (negative control) outcomes.

BMI was split into quintiles for mothers and fathers to assess for deviation from linearity in the association with MIH. The quintile variable was treated as both continuous and categorical in logistic regression models. We report p-values for a linear trend (Table S2) (Lim *et al*., 2022).

Sensitivity analysis defining cases as those that had affected FPMs and permanent incisors using the age 10 questionnaire and on complete cases (cases with no missing data on exposure, outcome, or confounders) investigated the influence of missing data (missing data reported in Table S1). Complete case outcomes were compared with the main analyses, which is reported in the supplementary data (Table S3). All supplementary analyses are available at https://doi.org/10.6084/m9.figshare.20337657.v1 (Lim *et al.,* 2022).

Results

Table 1 summarises the distributions of offspring, maternal and paternal characteristics of this study population. 5,536 offspring had completed the dental questionnaires and had at least one FPM tooth. The overall prevalence of offspring MIH was 5.4%, and 40 of the 297 (18.8%) MIH cases had both molars and incisors affected at age 10. The prevalence of dental trauma was 13.5%. Mean BMI was 22.3kg/m^2 and 25.1kg/m^2 for mothers and fathers, respectively. One fifth (21.4%) of mothers smoked during pregnancy and 31.6% of fathers smoked around the time of pregnancy. Most (73.5%) of mothers consumed any alcohol during pregnancy and 96.1% of fathers consumed any alcohol around the time of pregnancy.

In the confounder and other parent BMI-adjusted model (model 3), there was 4% greater odds of offspring MIH for every unit (1kg/m²) increase in maternal BMI (Odds Ratio (OR): 1.04, 95% CI: 1.00-1.08) (Table 2 and Figure 3A). In comparison, point estimates for paternal BMI were in the opposite direction (OR: 0.94, 95% CI: 0.89, 1.00). Negative control outcome analyses for maternal BMI and offspring dental trauma showed no difference in odds, with point estimates around the null (OR: 0.99, 95% CI: 0.96, 1.02) (Figure 3A and Table S2). Results of complete case analyses were broadly consistent (Table S4) (Lim *et al.,* 2022). Taken together, these results provide some evidence of an association between mean maternal BMI and offspring MIH with negative control analyses suggesting that the results are unlikely to be being driven by unmeasured confounders. Analyses of BMI quintiles compared linear and categorical models; whilst there was evidence for a linear trend for maternal BMI (p-value for per fifth increase = 0.04), this appeared to be driven by the highest quintile (OR for women in the highest BMI quintile versus the lowest: 1.61, 95% CI: 1.02, 2.60). In comparison, there was no evidence of a positive association for any of the paternal BMI quintiles and offspring MIH (Figure 3B).

Unadjusted associations showed maternal pregnancy smoking was associated with an increased odds of offspring MIH (OR: 1.41, 95%CI 1.06, 1.86) (Table 2). After adjusting for confounders, the association attenuated to the null (model 2: OR: 0.98, 95%CI: 0.68, 1.38; model 3: OR: 0.76, 95%CI: 0.46, 1.22). Point estimates for both negative controls, paternal smoking (model 3: OR: 1.21, 95%.CI: 0.82, 1.77). and offspring dental trauma (Table S2 OR: 1.20, 95%CI: 0.93, 1.53) (Lim *et al.,* 2022) showed positive associations, however confidence intervals spanned the null. Taken together, these results provide no evidence of an intrauterine effect of maternal smoking and offspring MIH, although results were imprecisely estimated. In complete case analyses (Table S3), all estimates for maternal smoking (including the unadjusted model) were in line with the confounder adjusted main analysis (Lim *et al.,* 2022).

Associations for alcohol consumption were largely imprecise and showed no clear evidence of an association in maternal (OR: 0.79, 95%CI: 0.56, 1.21) or paternal confounder and other parent adjusted models (OR: 1.04 95%CI: 0.41, 3.47) (Table 2). Similarly, negative control outcome analyses with dental trauma (Table S2) showed no clear evidence of an association with maternal alcohol consumption (OR: 0.94, 95%CI 0.75, 1.18) (Lim *et al.,* 2022).

Discussion

In this prospective cohort study, we found some evidence of a possible intrauterine effect between higher maternal pre-pregnancy BMI and offspring MIH. Additional investigations uncovered that this association appeared to be non-linear and may be driven by a threshold effect in the highest BMI quintile. We did not find similar associations in our negative control analyses, suggesting that

Table 1: Participant characteristics.

	All	*MIH*	*No MIH*
Offspring (N= 5536)			
N (%) MIH case (molars only)	297 (5.4)	297 (100.0)	
N (%) MIH sensitivity (molars and incisors)	40 (0.7)	40 (18.8)	
N (%) Dental trauma	621 (13.5)	0 (0.0)	621 (14.4)
Maternal			
Mean (SD) age (years)	29.5 (4.5)	29.2 (4.6)	29.53 (4.5)
Mean (SD) BMI (kg/m²)	22.3 (4.1)	22.9 (4.8)	22.28 (4.0)
N (%) Pregnancy Smoking			
Trimester 1	988 (18.2)	67 (23.0)	921 (18.0)
Trimester 2	782 (14.4)	57 (19.6)	725 (14.1)
Trimester 3	747 (15.1)	50 (19.5)	697 (14.9)
Any smoking	1,072 (21.4)	73 (27.3)	999 (21.0)
N (%) Pregnancy Alcohol			
Trimester 1	3,001 (55.6)	153 (53.3)	2,848 (55.7)
Trimester 3	1,074 (35.0)	47 (29.7)	1,027 (35.3)
Any alcohol	3,224 (73.5)	166 (70.9)	3,058 (73.7)
N (%) Parity	2,841 (53.0)	152 (52.8)	2,689 (53.0)
Education			
Low: None/ CSE	710 (13.3)	55 (19.7)	655 (12.9)
Medium: Vocational/ O/ A Level	3,713 (69.7)	184 (65.9)	3,547 (69.9)
High: Degree	910 (17.0)	40 (14.3)	870 (17.2)
Paternal			
Mean (SD) age (years)	31.6 (5.6)	31.3 (5.9)	31.57 (5.5)
Mean (SD) BMI (kg/ m²)	25.1 (3.2)	24.9 (3.2)	25.13 (3.2)
Any Pregnancy Smoking N (%)	1,344 (31.6)	71 (33.8)	1,273 (31.5)
Any Pregnancy Alcohol N (%)	4,219 (96.1)	205 (95.3)	4014 (96.1)
N (%) Education			
Low: None/ 2° or equivalent.	996 (19.1)	65 (24.3)	931 (18.8)
Medium: Vocational/ O/ A- level	3,043 (58.2)	146 (54.5)	2,897 (58.4)
High: Degree level	1,186 (22.7)	57 (21.3)	1,129 (22.8)

these results are unlikely to be explained by confounding. We found no robust evidence to suggest a causal intrauterine effect of maternal pregnancy smoking or alcohol consumption on offspring MIH, although these results were less precise. To our knowledge, this is the first study to use prospective data in large numbers using negative control analyses to explore possible maternal pregnancy risk factors for offspring MIH.

There are several potential explanations for why mothers with higher BMI may be more likely to have offspring with MIH. Firstly, FPMs develop *in utero*, therefore it is biologically plausible that maternal pre-pregnancy BMI (which is reflective of weight during early pregnancy) may influence MIH. Pre-pregnancy obesity provides an unfavourable environment for foetal development due to supply of nutrients crossing the placenta in deficit or overabundance (Leddy *et al*., 2008; Maffeis and Morandi, 2017). This may cause metabolic and physiological changes which alter growth and development (Leddy *et al.*, 2008; Maffeis and Morandi, 2017). A second explanation is that we have not fully accounted for confounders. However, our stringent confounder adjustments and negative control analyses do not support the presence of confounding. Finally, this finding could be a false positive (due to chance). As this is the first study to investigate pre-pregnancy BMI as a potential risk factor for MIH it cannot be compared with previous work. More research with larger studies is warranted to replicate the association with BMI and assess the effects of obese and severely obese World Health Organisation categories. If supported, our findings would have implications to further encourage healthy pre-pregnancy BMI in women trying to conceive.

We did not find evidence of an association between maternal pregnancy smoking and offspring MIH. Although unadjusted analyses associated offspring MIH in mothers who smoked, the odds were attenuated close to null after adjusting for confounders. However, these associations were imprecise, therefore we cannot simply reject an association. As these analyses had limited statistical power, negative control explorations were less meaningful. Our findings support findings of systematic reviews, that there is little evidence for an association between pregnancy smoking

Table 2: Negative control exposure analyses showing associations between maternal and paternal exposures (pre-pregnancy BMI, pregnancy smoking and alcohol consumption) with offspring MIH.

	Model 1 *OR (95% CI)*	*Model 2* *OR (95% CI)*	*Model 3* *OR (95% CI)*
BMI			
Maternal BMI	1.03 (1.00-1.06)	1.04 (1.00-1.07)	1.04 (1.00-1.08)
N Total	4830	3582	2698
N Case	252	184	134
Paternal BMI	0.97 (0.92-1.01)	0.96 (0.91-1.01)	0.94 (0.89-1.00)
N Total	3993	2968	2713
N Case	202	142	128
Smoking			
Maternal Smoking	1.41 (1.06, 1.86)	0.98 (0.68, 1.38)	0.76 (0.46, 1.22)
N Total	5017	4025	3184
N Case	267	210	151
Paternal Smoking	1.11 (0.82, 1.48)	1.16 (0.81, 1.64)	1.21 (0.82, 1.77)
N Total	4250	3270	3061
N Case	210	155	144
Alcohol			
Maternal Alcohol	0.87 (0.65-1.17)	0.87 (0.64-1.18)	0.79 (0.56-1.12)
N Total	4384	4025	3288
N Case	234	210	155
Paternal Alcohol	0.83 (0.45-1.70)	1.03 (0.48-2.67)	1.04 (0.41-3.47)
N Total	4391	3270	2666
N Case	215	155	127

Results of univariable and multivariable logistic regression analyses shown as odds ratio (OR) and 95% Confidence intervals (95% CI). Model 1 = unadjusted, Model 2 = confounder adjusted: age, education, parity (maternal), pregnancy smoking (BMI and alcohol analyses) and alcohol consumption (BMI and smoking analyses), Model 3 = adjusted for the same confounders as model 2 and additionally for the other parent's exposure during/around pregnancy.

on offspring MIH (Fatturi *et al*., 2019; Silva *et al*., 2016). A recent well-powered case-control study, not included in these reviews, associated pregnancy smoking with offspring MIH (Lee *et al*., 2020). However, the exposure data were collected retrospectively, allowing the risk of information bias (Sutton-Tyrrell, 1991). Moreover, the analyses in that study did not account for the range of confounders included here (e.g. parity and alcohol consumption), which contributed to the full attenuation of the smoking effect on MIH.

Point estimates for maternal alcohol consumption were in the protective direction on offspring MIH were imprecise. Therefore, no clear evidence of an association was found, and negative control explorations were not required to explore residual confounding. A protective association seems unlikely as there is no plausible biological explanation. Results from other studies are conflicting and lack statistical power (Fatturi *et al.*, 2019), therefore the effects of alcohol consumption on MIH remains unclear.

A key strength of this study is the use of prospective cohort data, which limits recall bias. Other strengths include adjusting for a wide range of prospectively measured confounders, and the use of negative control analyses to detect residual confounding, which no previous study has accounted for. Taken together, these steps improved robustness. An important limitation is our definition of MIH, "6-year molars that came through brown" at age 7. This may be oversimplistic in comparison to the gold standard EAPD classification (Weerheijm, 2003), and prone to misclassification due to the lack of a clinical diagnosis. However, this will be non-systematic and would, at worst, attenuate the magnitude of any association. We intended to prevent misclassification by conducting a sensitivity analysis, including cases with affected molars and incisors. However, with the small sample (40/297, 18.8%), additional analyses would not have been particularly informative. The reported prevalence of MIH in our study population was 5.4%; this is at the lower end of the reported range and may be underrepresented. This may be because our cases of MIH were not clinically diagnosed, so missing mild cases. Formal validation of the self-complete questionnaire by clinical examination was not conducted and unfortunately is no longer feasible. Other limitations include self-reporting of data which may have resulted in underreporting of smoking and alcohol consumption; however, this would be expected to weaken any true effect of these exposures, rather than create false positives.

It is also possible that we did not account for all potential confounders in the maternal analyses, for example other pregnancy medical comorbidities such as anaemia and high blood pressure. However, we addressed any residual confounding in our paternal negative control

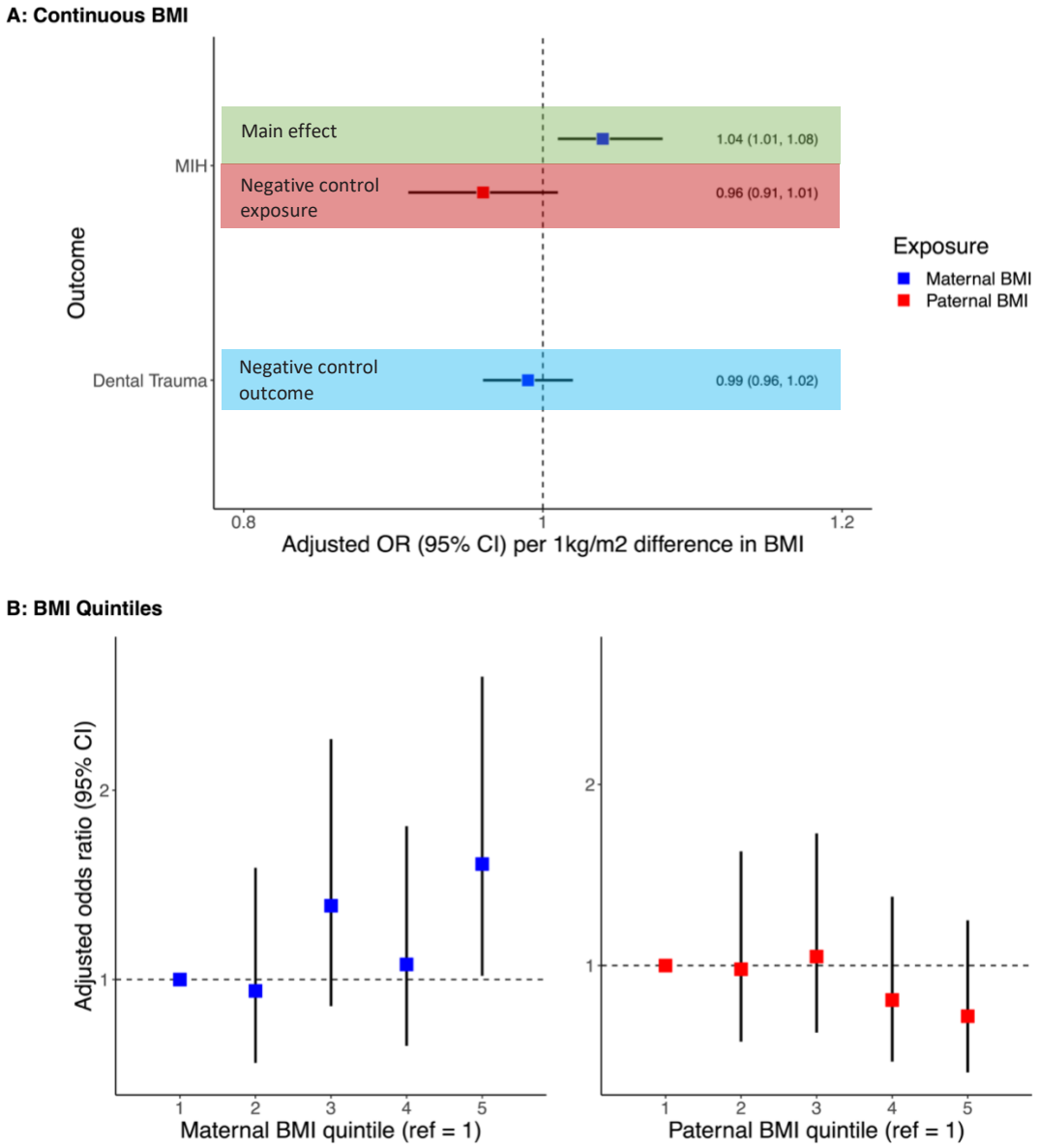

Figure 3. *BMI Analyses.*
Confounder and other parent body mass index-adjusted associations (model 3) for maternal and paternal BMI and offspring molar-incisor hypomineralisation. Confounders adjusted for include age, parity (maternal), education, pregnancy smoking, pregnancy alcohol consumption and the other parent's BMI. A shows the odds ratio and 95% confidence intervals of offspring MIH for a 1-unit (1kg/m2) difference in maternal BMI (blue) and paternal BMI (red). B shows the confounder and other parent BMI-adjusted associations for maternal (red) and paternal BMI (blue) split into quintiles (fifths) and offspring MIH. Results are odds ratio and 95% confidence intervals for BMI quintile and offspring MIH in comparison to BMI quintile 1.

analyses. An important limitation of this method is that if an association is found with the presence of unmeasured confounding, we cannot identify the confounder (Sanderson *et al*., 2018; Lipsitch *et al*., 2010).

This study adds to the limited evidence regarding modifiable maternal risk factors on offspring MIH. Previous research primarily focuses on non-modifiable risk factors such as maternal and childhood illnesses (Fatturi *et al*., 2019; Silva *et al*., 2016), for which intervention is difficult. As the aetiology of MIH is likely to be multifactorial, future research exploring modifiable maternal risk factors, such as BMI, smoking and alcohol consumption, may enable preventative strategies or remove stigma from mothers of children with MIH. To improve comparability and accuracy, future studies could use prospective data with larger samples, clinically diagnose MIH using the EAPD criteria (Weerheijm, 2003), adjust for relevant confounding variables and use epidemiological methods to improve causality (eg. negative control analyses). As large enough samples may be difficult to obtain, cross-cohort studies may be an alternative approach to gain sufficient statistical power.

In conclusion, this study showed some evidence of an intrauterine effect of higher maternal BMI on offspring MIH. We did not find robust evidence for an effect of maternal pregnancy smoking or alcohol consumption on risk of offspring MIH. Further studies with larger numbers utilising prospectively collected data with advanced epidemiological methods and formal MIH diagnoses are required to replicate and further investigate these findings. Furthermore, exploring possible mechanisms that link the pregnancy environment to offspring MIH could identify targets for interventions for its prevention.

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Conflict of interest

The authors declare no conflict of interest.

Data availability

The data that support the findings form this study are available from the ALSPAC cohort (http://www.bristol.ac.uk/alspac/) but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available upon reasonable request and with permission of ALSPAC executive.

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