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Article

Association between Exposure to Metals during Pregnancy, Childhood Gut Microbiome, and Risk of Intestinal Inflammation in Late Childhood

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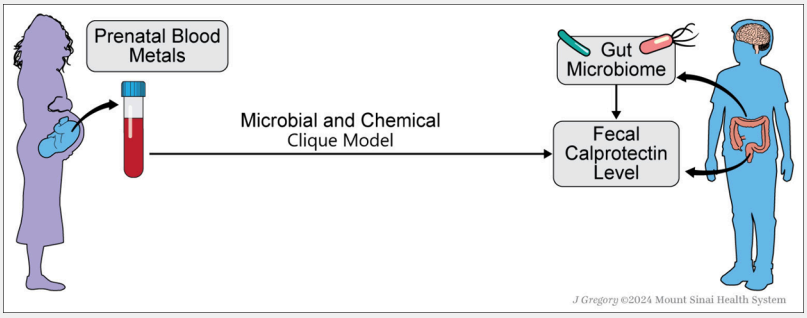

ABSTRACT: Alterations to the gut microbiome and exposure to metals during pregnancy have been suggested to impact inflammatory bowel disease. Nonetheless, how prenatal exposure to metals eventually results in long-term effects on the gut microbiome, leading to subclinical intestinal inflammation, particularly during late childhood, has not been studied. It is also unknown whether such an interactive effect drives a specific subgroup of children toward elevated susceptibility to intestinal inflammation. We used an amalgamation of machine-learning techniques with a regression-based framework to explore if children with distinct sets of gut microbes and certain patterns of exposure to metals during pregnancy (metal−microbial clique signature) had a higher likelihood of intestinal inflammation, measured based on fecal calprotectin (FC) in late childhood. We obtained samples from a well-characterized longitudinal birth cohort from Mexico City (n = 108), Mexico. In the second and third trimesters of pregnancy, 11 metals were measured in whole blood. Gut microbial abundances and FC were measured in stool samples from children 9−11 years of age. Elevated FC was defined as having FC above 100 μg/g of stool. We identified subgroups of children in whom microbial and metal−microbial clique signatures were associated with elevated FC (false discovery rate (FDR) < 0.05). In particular, we found two metal−microbial clique signatures significantly associated with elevated FC: (1) low cesium (Cs) and copper (Cu) in the third trimester and low relative abundance of *Eubacterium ventriosum* (OR [95%CI]: 10.27 [3.57,29.52], FDR < 0.001) and (2) low Cu in the third trimester and high relative abundances of *Roseburia inulinivorans* and *Ruminococcus torques* (OR [95%CI]: 7.21 [1.81,28.77], FDR < 0.05). This exploratory study demonstrates that children with specific gut microbes and specific exposure patterns to metals during pregnancy may have higher fecal calprotectin levels in late childhood, denoting an elevated risk of intestinal inflammation.

KEYWORDS: *exposome, metals, machine learning, microbiome, environmental epidemiology, intestinal inflammation*

INTRODUCTION

Preclinical and early life environmental signatures of inflammatory bowel disease (IBD), an immune-mediated disease of the intestinal tract, are increasingly being recognized.[1] Recent literature suggests that preclinical IBD can occur at least two years prior to IBD diagnosis and could be identified using

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subclinical inflammation markers.[2] Identifying preclinical markers of IBD can help with diagnosis, treatment, and disease monitoring.[3] Biomarkers of preclinical IBD include intestinal inflammation and distinct gut microbiome perturbations.[1] Raygoza Garay et al. reported that, among first-degree relatives of individuals with Crohn's disease (CD) (a subtype of IBD), a microbiome risk score (MRS) based on specific taxonomic features was predictive of subsequent CD diagnosis.[4] The group also reported elevated fecal calprotectin (FC) as a marker of subclinical intestinal inflammation preceding CD diagnosis.[1] Offspring of women with IBD possess distinct microbiome features with loss of bacterial diversity and elevated FC compared to offspring of women without IBD.[5,6] This indicates that programming of the gut microbiome, intestinal health, and IBD risk in childhood may occur during the prenatal period. However, the impact of gut microbiome signatures on preclinical intestinal inflammation has not been well characterized. Mapping these relationships is pertinent to understanding IBD risk factors and pathways and for prediction and prevention.

Metal exposures during the prenatal period have been hypothesized to be particularly important in the programming of later gut microbiome function, intestinal inflammation, and IBD risk.[7,8] Transition metals (iron, zinc, copper, manganese, molybdenum, etc.) are biologically necessary for humans as part of the immune system and are used during inflammation to fight infections, both by limiting bacterial access to these essential metals and by employing metal toxicity to kill invading bacteria.[9,10] This process of controlling infection through immune-mediated metal availability and toxicity is called nutritional immunity.[11] As part of nutritional immunity, neutrophils produce and secrete calprotectin, which serves as a high-affinity manganese/zinc binding protein, limiting the availability of the metals to bacteria within the gut microbiome.[12] Metal withholding by calprotectin is likely to have a negative impact on both commensal and pathogenic bacteria.[13] Both high and low essential metal exposures have been shown to influence the gut microbiome diversity and have been correlated with gut inflammation. Likewise, toxic metals (e.g., mercury and lead) have been shown to alter gut microbiome composition and function.[14−16] Moreover, a growing body of literature suggests that prenatal metal exposures can alter the gut microbial composition and metabolic functions later in childhood.[17] This may be partly due to prenatal programming of nutritional immune function,[18] which suggests that certain nutrients and elemental exposure during the prenatal or perinatal period may interact and possibly shape how the body reacts to certain external perturbances and modifies microbial diversity.[19] Prenatal metal levels have also been associated with IBD risk later in life.[20] However, little is known regarding the relationship among prenatal metal exposures, the gut microbiome, and intestinal inflammation, especially during childhood.

Environmental studies suggest that important biochemical interactions occur among specific bacterial taxa, varying from a few to many bacterial species.[21] Early life metal exposures may influence these interactions.[22] However, the effect of these interactions may be more pronounced in certain subgroups of the sample.[22] Therefore, similar to the conceptual framework of "genetic signature", which can be present, particularly in a specific subpopulation, we introduce the concept of a "metal−microbial clique signature". We define this as a signature that can be characterized by metal concentrations and microbial abundances, and only a certain subgroup of children with specific patterns of metal exposure during pregnancy and microbial abundance above or below certain thresholds in late childhood will have these definite signature patterns. We hypothesize that children with specific metal−microbial cleavage signatures will have a higher risk of preclinical intestinal inflammation. Such a clique signature, therefore, conceptually differs from individual and metal mixture associations, where every individual in the sample has simultaneous exposures but only a specific subgroup is exposed to a clique signature. Identifying such clique signatures is technically challenging since simultaneously searching for multiordered combinations and estimating thresholds to create subgroups are computationally very intensive. For example, choosing two-ordered or three-ordered combinations from a set of 100 microbes and 11 metals yields more than 5×10^3 and 2×10^5 combinations to choose from. On top of that, there is additional complexity in finding thresholds. Therefore, parsing through such vast combinations is substantially challenging if classical statistical tools are used solely. Hence, the use of machine learning techniques not only provides a way forward but also provides a framework for precision environmental health. In this article, we explore whether children with certain metal−microbial clique signatures have a higher risk of developing intestinal inflammation in late childhood using an ensemble of machine-learning tools with a regression-based framework.

MATERIALS AND METHODS

Study Population

This study leverages data from the Programming Research in Obesity, Growth, Environment, and Social Stressors (PROGRESS) cohort in Mexico City, Mexico, an ongoing longitudinal birth cohort.[23,24] The PROGRESS cohort enrolled 948 pregnant women and their offspring and is designed to examine associations between early life environmental toxicant exposures (i.e., metals) and pediatric health. Recruitment started in July 2007. Pregnant women were enrolled in the Mexican Social Security System at the beginning of the study. Participants completed study visits in the second and third trimesters, and mothers and offspring were followed approximately every two years from birth to adolescence. Study visits included surveys, physical exams, biological sample collection, and psychological and behavioral testing. Approximately 600 mother−child pairs are actively followed by surveys, physical exams, and psychological and behavioral assessments with children currently 14−16 years old. Blood and urine samples were collected when the children were 4, 6, 8, and 10 years of age (on average). As a substudy based on this cohort, stool sampling occurred at the study visit when children were between 8 to 11 years of age in a convenience subsample of the cohort (n = 123); however, 108 had complete outcome data available. Before the stool collection, all children with a history of antibiotic usage in the past month of stool sample collection were excluded.

Metal Measurement

During the second and third trimesters of pregnancy, whole blood samples from mothers were used to measure metal concentrations. As noted in our previous studies, in the "Trace Metals Laboratory of the Icahn School of Medicine at Mount Sinai", multiple metal concentrations, including arsenic (As), copper (Cu), chromium (Cr), cadmium (Cd), selenium (Se), antimony (Sb), lead (Pb), cesium (Cs), zinc (Zn), manganese (Mn), and cobalt (Co), were determined using the "Agilent 8800 ICP Triple Quad (ICP-QQQ) in MS/MS mode". Further, all measurements were taken in five replicates and reported as an average, and the quality control standards were recovered at 90−110%. For laboratory elemental analysis, quality assurance, and quality control procedures, the following measures were undertaken: (1) initial verification standards, (2) analyses of calibration standards in the range

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of 0.001 to 50 ng/mL, and (3) continuous calibration verification standards.[25,26]

Gut Microbiome Data Collection and Further Processing

Details on stool sample collection and subsequent analysis are presented in our previous works.[17,22,27] Briefly, at the 8−11 year visit, stool samples were collected from the children. Samples were self-collected by participants with help from their parents, as required. Whole stool samples were processed and aliquoted following the FAST protocol at the ABC Hospital in Mexico City.[28] Frozen samples for further analysis were sent to the Microbiome Translational Center at Mount Sinai. Next, samples were processed and sequenced in two batches, $n = 73$ and $n = 50$, depending on the time of receipt of the stool samples. Using the NEBNext DNA Library Prep kit, shotgun metagenomic sequencing was performed and eventually sequenced on an Illumina HiSeq. We used Trimmomatic to trim the quality of the sequencing reads, and human reads were removed by mapping to a reference with bowtie2.[29,30] To determine microbial taxonomy at the species/strain level, the remaining reads were processed using MetaPhlAn2, StrainPhlAn, and HUMAnN2 to profile microbial gene pathways.[31−33]

Fecal Calprotectin Measurements

Aliquots from the same stool samples were weighed to 40 mg for fecal calprotectin (FC) measurements using a CALPROLAB Calprotectin enzyme-linked immunosorbent assay (ELISA) kit (Svar Calpro, Lysaker, Norway). All procedures were performed as previously described.[6] We used high FC as the outcome, defined as ≥100 μg/g of stool.[34] Previous reviews have shown that, for children, a cutoff of 100 μg/g for fecal calprotectin provides almost 100% sensitivity and 90% specificity in detecting IBD vs non-IBD cases.[35] Therefore, we will use this binary outcome for clinical relevance and an improved interpretation of our findings.

Covariates

Covariates included in the subsequent analyses were (1) maternal socio-economic status (SES) during pregnancy, (2) maternal body mass index (BMI) during pregnancy, (3) maternal age at birth, (4) the child's age at the time of stool sample collection, (5) child sex, and last (6) the microbiome analysis batch. Measured height and weight were used to calculate BMI. As noted in earlier papers, "the SES during pregnancy was assessed using the 1994 Mexican Association of Intelligence Agencies Market and Opinion. Families were classified into six levels based on 13 questions about household characteristics. In the study, most families belonged to the low-middle SES category; thus, we condensed all the six categories into three categories: low, mid, and high".[22,36] For simplicity and due to smaller sample sizes, we kept the covariates minimal.[17]

Statistical Methods

We conducted all analyses in R (version 4.2.3). We followed a similar analysis strategy as our previous work and reiterated details for replication.[17] For univariate analyses, we used covariate-adjusted logistic regressions. The association estimates for metals were presented through forest plots, whereas we used volcano plots for the relative bacterial abundances. The metal concentrations and relative abundances of the microbial taxa were transformed into quartiles to ward off influences of outliers and for homogeneity in variance. The metal concentrations below the limit of detection were therefore assigned the lowest quartile values. Although most exposures had a slight right skewness in their distributions, such skewness could possibly bias the standard errors in regression analysis and therefore bias the asymptotic normality of the p-value. To combat this, we conducted normality-agnostic robust permutation tests (as sensitivity analyses) to verify the p-values of the reported associations. The unadjusted p-values were corrected by using the false discovery rate (FDR) procedure. Any missing values in covariates were imputed using the predictive mean matching algorithm as implemented through the MICE R package.[37] The main analyses were conducted on the imputed data set. To ensure that there is no substantial batch effect in the microbiome data processing, first, we conducted a detailed principal component analysis on the β diversity metrics by batch; second, we only used those microbial taxa that had at least 5% relative abundance in both batches; and third, we adjusted for the batch indicator in all of our models.

The downstream analysis identifies combinations of prenatal metal exposures and gut microbes and their thresholds, eventually creating the metal−microbial clique signatures. As an example, consider a clique signature of microbe X and metals Y and Z, denoted by X+/Y−/Z+, which implies a subgroup of the sample with a higher relative abundance of X, a lower concentration of metal Y, and a higher concentration of metal Z. Therefore, a clique signature in this analysis is represented as a binary variable, characterizing a specific subgroup of children with particular patterns of prenatal metal exposures and microbial relative abundances. We used an interpretable machine-learning algorithm called the "repeated hold-out signed-iterated Random Forest" (rh-SiRF) and merged it with a regression-based inference framework, where the relative abundances of the gut microbial taxa and prenatal metal exposures during the second and third trimesters were treated as predictors, and elevated FC as the outcome.[38−40] Previously, after controlling for the overall environmental chemical mixture effect, the rh-SiRF algorithm was used to find highly ordered interactions among chemical exposures. The rh-SiRF algorithm combines (1) a weighted random forest algorithm that iteratively assigns higher weights on predictors with better discriminatory performance and (2) within the forest searches for combinations of nodes (predictors) that co-occur together in the generated trees. Among the bootstrapped iterations and throughout the forest, if a certain combination of predictive nodes co-occurs multiple times, then the rh-SiRF algorithm chooses such a combination and provides statistics to quantify the strength of its occurrence. Finally, using a "threshold finding algorithm", the multiordered combinations were converted into binarized clique signatures.

The rh-SiRF model was fitted in three stages to create cliques of metals, cliques of microbes, and cliques of metals−microbes: (1) first, two separate models were fitted on a subset of 70% data ($n = 76$), one with prenatal metal concentrations as the predictors and the other with microbial relative abundances as the predictors. (2) Next, we extracted the top 5% of the most commonly occurring combinations from both models. The cutoff of 5% was chosen to obtain, at most, a total of 10 stable clique features. We chose a conservative cutoff since the higher the cutoff, the higher the possibility of including overfitted cliques for the next stage of modeling. (3) Lastly, we again fitted two rh-SiRF models using the training data set ($n = 76$) with the top 5% most stable metal combinations and all microbial abundances as predictors and the other with the top 5% most stable microbial combinations and all metal concentrations as predictors. The aim of this third step was to identify all possible metal−microbial clique signatures of a higher order. With the small sample size, this algorithm could not often find more than two ordered cliques; therefore, in the third stage, we included both models to create three-ordered clique signatures. The sole individual metal combinations and microbial combinations were chosen from the first stage, whereas the metal−microbial combinations were chosen from the third stage. Each model was repeated at least 500 times with 200 bootstrap iterations, ensuring that the often occurring combinations are not just an over fitted combination; instead, they must occur multiple times throughout the forest to be picked up by the algorithm. Further, each machine-learning model was fitted on a continuous log-transformed calprotectin value to combat overfitting. In contrast, the eventual downstream regression analyses were conducted on binarized FC. Finally, we converted these combinations into predictive clique signatures using a threshold-finding algorithm for the following regression analysis stage. We used the whole data set (instead of 30% test data for the regression analysis) because the regression estimates would not be robust with a mere sample size of $n = 32$, and none of the asymptotic properties would kick in. Although this is not the ideal scenario, training on 70% of the data and regression (testing) on the whole data create some form of separation and help to instill the asymptotics in p-values with better coverage for the 95% CIs.

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Table 1. Characteristics of the Study Population

variable[a]	overall (n = 108)	low fecal calprotectin (<100 μg/g)	elevated fecal calprotectin (≥100 μg/g)	p-value[b]
child sex				0.83
male, n (%)	62 (57.41%)	43 (56.58%)	19 (59.38%)	
female, n (%)	46 (42.59%)	33 (43.42%)	13 (40.63%)	
maternal SES in pregnancy				0.79
low, n (%)	59 (54.63%)	43 (56.58%)	16 (50.00%)	
mid, n (%)	38 (35.19%)	25 (32.89%)	13 (40.63%)	
high, n (%)	11 (10.19%)	8 (10.53%)	3 (9.38%)	
maternal age in years at offspring birth, mean (SD)	28.31 (5.65)	28.83 (5.86)	27.09 (5.00)	0.21
maternal BMI (kg/m^2), in pregnancy, mean (SD)	26.90 (4.37)	27.02 (4.42)	26.63 (4.31)	0.82
child age in years at stool sample collection, mean (SD)	9.67 (0.88)	9.71 (0.87)	9.59 (0.91)	0.32
Shannon α diversity index, mean (SD)	2.35 (0.39)	2.38 (0.37)	2.29 (0.44)	0.29
2nd trimester Mn concentration (μg/L), mean (SD)[c]	14.53 (5.43)	15.23 (5.89)	12.88 (3.70)	0.07
2nd trimester Cr concentration (μg/L), mean (SD)[c]	0.77 (1.11)	0.67 (0.89)	1.00 (1.52)	0.42
3rd trimester Cs concentration (μg/L), mean (SD)[c]	2.64 (0.85)	2.63 (0.82)	2.66 (0.92)	0.90
3rd trimester Cu concentration (μg/L), mean (SD)[c]	1621.35 (275.50)	1645.94 (301.94)	1562.97 (190.85)	0.38

[a]SES, socioeconomic status; BMI, body mass index; Mn, manganese; Cr, chromium; Cs, cesium; Cu, copper. [b]p-values were obtained using Fisher's exact test or Wilcoxon rank sum test as required. [c]Concentrations of all metals in the second and third trimesters are provided in the Supporting Information

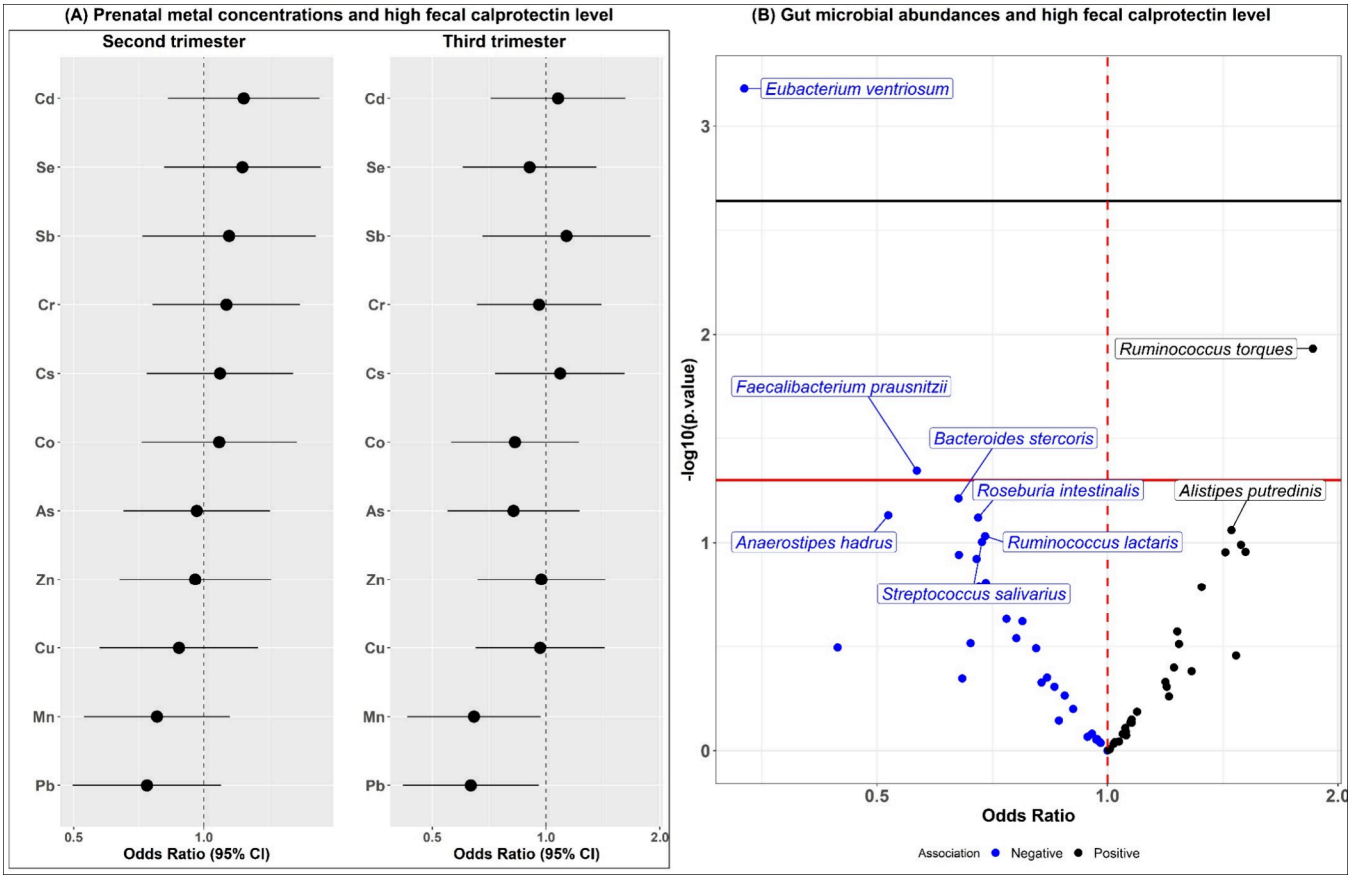

Figure 1. Prenatal metal exposures, gut microbial relative abundances, and elevated fecal calprotectin level. (A) Prenatal metal exposures and odds of high fecal calprotectin (≥100 μg/g) presented through forest plots. (B) Microbial relative abundance and adjusted odds of high fecal calprotectin. Part (A) shows the individual associations (adjusted odds ratios (OR) and 95% CI) between prenatal metal exposures at second and third trimesters and high fecal calprotectin (≥100 μg/g). The metal concentrations were transformed into quartiles for robustness. Part (B) demonstrates the OR for associations between microbial relative abundance and elevated fecal calprotectin. The p-values and the ORs are plotted through a volcano plot. The red horizontal line denotes the log(p-value) at 0.05, the nominal p-value. In contrast, the black horizontal line denotes the log(p-value) at 0.002, the multicomparison error adjusted p-value.

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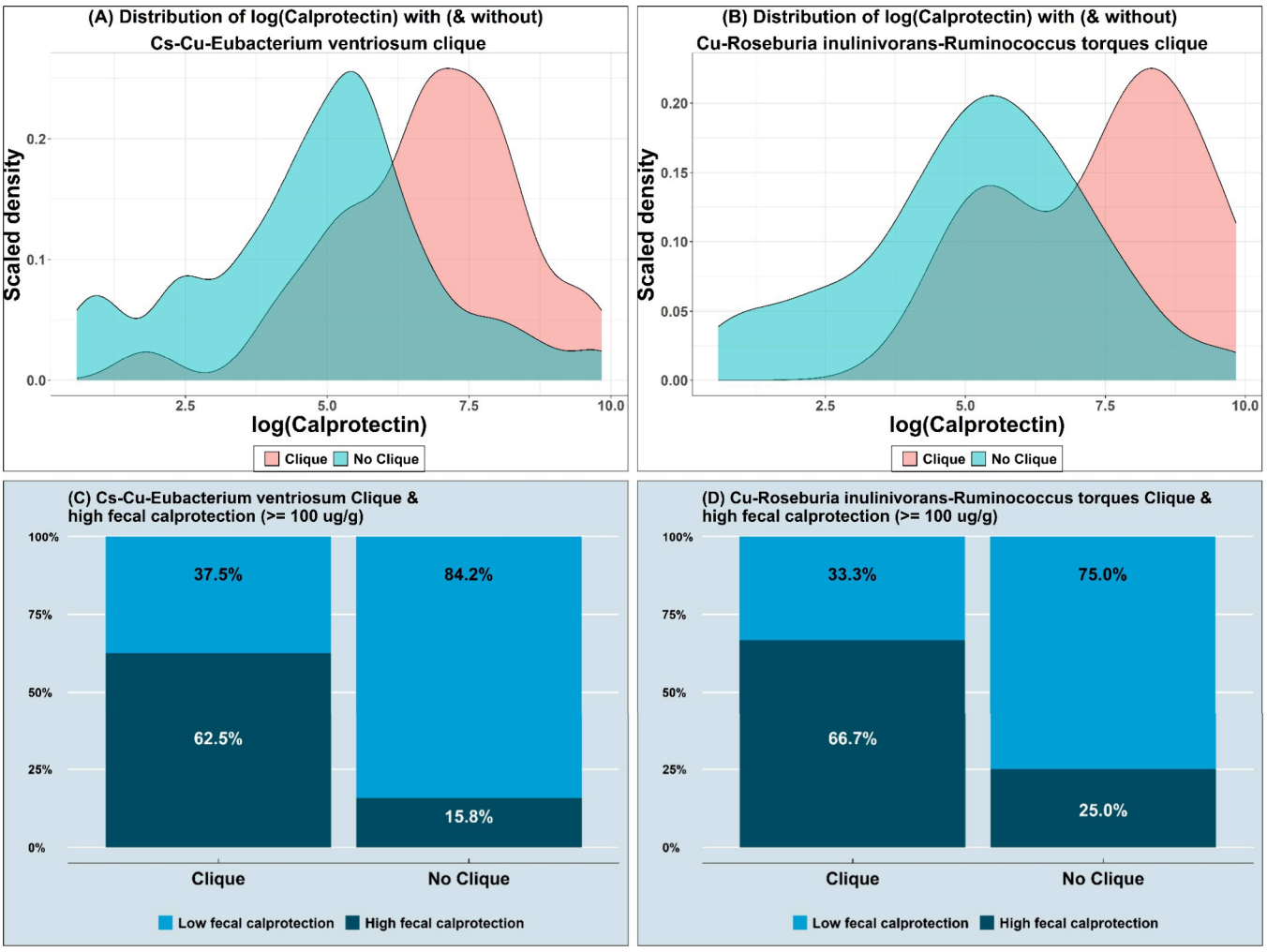

Figure 2. Distribution (density plot) of fecal calprotectin (continuous and binary) over subgroups of children with (or without) metal−microbial clique signatures. (A and B) The density plots of log-transformed calprotectin levels for subgroups of children with and without metal−microbial clique signatures. (C and D) Similar distributions through stacked bar plots for binary calprotectin levels.

Sensitivity Analysis

We conducted multiple sensitivity analyses to ensure the robustness of our results. First, we adjusted the regression models for the clique signature association analyses with the Shannon α diversity index. Second, to understand the impact of threshold choices on the directionality of the associations while constructing the clique signatures, we deliberately increased and decreased each threshold by 10 percentiles and refitted the models. Third, to ensure that the p-values obtained from the main regression-based metal−microbial clique models were robust, we conducted randomization with a permutation test, where we randomly permuted each of the outcomes 10^5 times and estimated robust p-values. Fourth, we repeated the main analysis using continuous calprotectin values and reported the standardized and scaled β estimates. Fifth, we repeated the main using a log-binomial model to report relative risks instead of odds ratios. Sixth, and most importantly, we presented the regression analysis for metal−microbial cliques on the 30% (n = 32) test data to highlight the robustness of the result.

RESULTS

Table 1 shows the characteristics of the study population. Among the 108 participants, there were more male children than female children (57% and 43%, respectively). The average age at stool sample collection was between 9 and 10 years; most children belonged to the mid-to-lower SES group. The distribution of prenatal metal concentrations and their corresponding percentage above the detection limits are presented in Table S1. The median (IQR) of FC was 43.9 (91.8) μg/g. Both the α (measured by Shannon index) and β diversities had almost null associations with the elevated FC (α diversity, OR = 0.91 and p-value = 0.2; β diversity, F = 1.39 and p-value = 0.17). In Table 1, we only included the concentrations of Mn, Cr, Cs, and Cu since these metals eventually formed metal cliques and metal−microbial cliques in the later stage of the analysis.

The results of the covariate-adjusted analyses for individual prenatal metals and gut microbial relative abundance are represented in Figure 1. The adjusted odds of elevated offspring FC by maternal blood metal levels varied by trimester (Figure 1A). Increased relative abundance of *Eubacterium ventriosum* and *Faecalibacterium prausnitzii* was associated with lower odds of elevated FC (OR [95%CI]: 0.33 [0.18,0.63], p-value < 0.001 and 0.56 [0.32,0.99], p-value < 0.05, respectively), while increased relative abundance of *Ruminococcus torques* was associated with higher odds of elevated FC (OR [95%CI]: 1.15 [1.85,2.99], p-value < 0.05, Figure 1B). Only the FDR-adjusted p-value for *E. ventriosum* was lower than 0.05.

We identified multiple two- and three-component metal, microbial, and metal−microbial clique signatures associated

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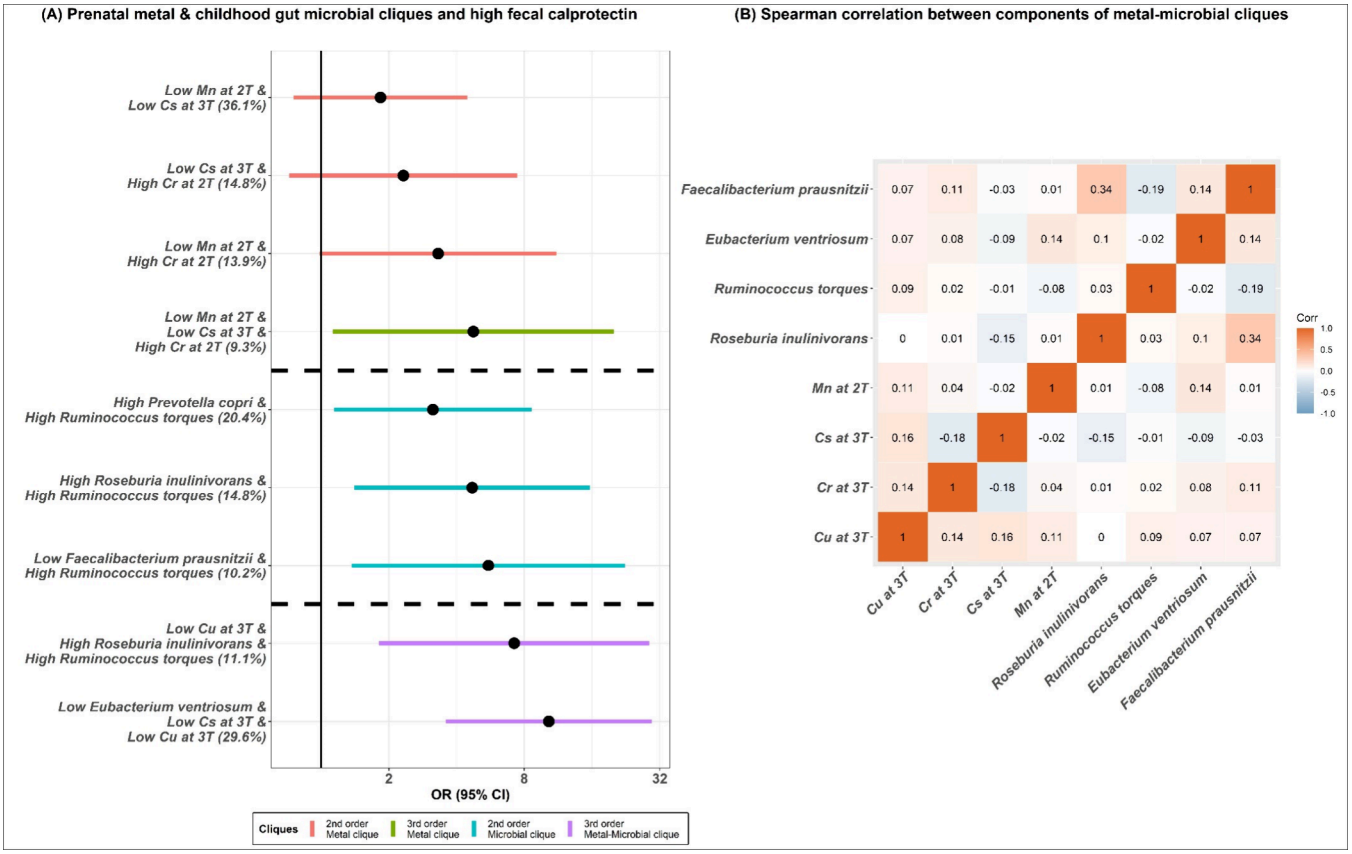

Figure 3. Metal−microbial clique signatures, odds of high fecal calprotectin, and the Spearman correlation between components of metal and microbial cliques. (A) The associations with metal and microbial clique signatures. The top rows (red, green) denote second- and third-order metal clique signatures, the middle rows (blue) denote microbial clique signatures, and the bottom rows (purple) denote the metal−microbe clique signatures. The sample proportion of children for each clique is denoted within brackets. The names of the clique signatures are noted on the *Y*-axis of (A). (B) The Spearman correlation between variables that constitute the clique signatures. 2T, second trimester; 3T, third trimester.

with elevated FC. However, the major metal−microbial clique signatures consisted of (1) low concentrations (below the 70th and 80th percentile of the sample) of Cs and Cu in the third trimester and low relative abundance of *E. ventriosum* (below the sample median) and (2) low concentration (below sample median) of Cu in the third trimester and high relative abundances of *Roseburia inulinivorans* and *R. torques* (both above the 60th percentile of the sample). Figure 2 shows the distribution of continuous log-transformed calprotectin levels and the binary high FC level (≥100 μg/g) for subgroups of children with and without the metal−microbial clique signatures. All the illustrative figures show that subgroups of children with any metal−microbial clique signatures were significantly more likely to have higher fecal calprotectin levels.

Figure 3A demonstrates associations across metal, microbes, and metal−microbial clique signatures. As illustrated by Figure 3, the strongest associations were observed among the metal−microbial clique signatures. We identified two subgroups of children (29.6% and 11.1% of the sample) characterized by the clique signatures Cs−Cu−*E. ventriosum* and Cu−*R. inulinivorans*−*R. torques* with significantly elevated FC in late childhood (OR [95%CI]: 10.27 [3.57,29.52], p-value < 0.0001 and OR [95%CI]: 7.21 [1.81,28.77], p-value < 0.01, respectively). Except for the associations with metal clique signatures, all associations with microbial and metal−microbial clique signatures had FDR-adjusted p-values below 0.05. The Spearman correlation between these two clique signatures was minimal (correlation = −0.04), indicating the detection of mutually exclusive subgroups. Similarly, the correlation across variables did not play a significant role in constructing metal−microbial clique signatures (Figure 3B).

The sensitivity analyses illustrated the robustness of the main results. Associations remained consistent after further adjustment for the Shannon α diversity index. We conducted two additional sensitivity analyses, where each threshold of the clique signature components was separately (1) increased and (2) decreased by 10 percentiles; however, the directionalities of all the major remained unchanged (Figures S1A and S1B). The permutated randomization-based p-values for the metal−microbial clique signatures remained as low as the model-based asymptotic p-values (p-value < 10^5). There were less than 5% missing values in a few covariates, and results did not alter significantly when the analysis was repeated in the nonimputed data set. The results for the associations with continuous calprotectin values are presented in Figure S2. The directionality of the associations remains unaltered, with the associations for metal−microbial cliques remaining significant. We also presented relative risk estimates instead of odds ratio for Figure 3A in Figure S3. Lastly and most importantly, we presented the regression analysis for metal−microbial cliques on the 30% (n = 32) test data (Figure S4). As expected, the directionality of the associations for metal−microbial clique associations remains unaltered (with one of them remaining statistically significant), while due to the small sample size, the 95% CIs are very broad. Therefore, this validates that the associations observed from the whole data analysis are not merely due to overfitting.

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DISCUSSION

Using a novel machine-learning approach, this pilot analysis investigated the effect of maternal metal exposure during pregnancy and the offspring's fecal microbiome on elevated intestinal inflammation in late childhood. We demonstrated that interactions among bacterial taxa in childhood and specific metals during pregnancy are associated with an elevated FC in otherwise healthy children. Our findings indicate that early life exposure to metals and their consequent interaction with the gut microbiome could intricately shape children's biological programming of gastrointestinal inflammatory processes. The use of an ensemble machine-learning approach allowed us to uncover previously unknown interactions among prenatal metal exposures, offspring fecal microbial features, and their effect on intestinal inflammation in children. To the best of our knowledge, this is the first study to report on microbial and metal−microbial interactions leading to subclinical intestinal inflammation. We also delineate specific clique signatures identifying children with higher susceptibility to worsening intestinal inflammation, highlighting the potential for prediction and prevention opportunities in late childhood. Our approach provides a framework for a precision environmental health approach.

The bacterial taxa that are of importance highlighted in our study have previously been associated with IBD risk. Specifically, *E. ventriosum* and *F. prausnitzii* have shown inverse associations with IBD and MRS,[5] respectively, while *R. torques* have been linked to MRS, a known predictor of CD onset.[4] These findings are consistent with perturbations observed in a multiomic analysis of 132 individuals.[41] Together, these data suggest that specific combinations of bacterial taxa may play a role in intestinal inflammation and IBD risk, and our findings highlight that when present as clique signatures, specific combinations of taxa may lead to intestinal inflammation as early as childhood. Using gut microbiome taxonomic features and fecal calprotectin may also help predict future risks of IBD, thereby developing a framework for prediction and prevention.

Additionally, maternal metal levels during pregnancy and their relation to inflammation and IBD remain unclear. A pilot study analyzing deciduous teeth indicated higher metal absorption levels (Cu, Zn, Cr, and Pb) among IBD cases compared to controls during different early life periods.[20] While these specific metals have been linked to IBD, further investigation is warranted to elucidate their role, specifically their long-term effect on childhood. Moreover, our findings of Cu and Cs concentrations align with previous evidence associated with inflammatory responses. Human and rodent models suggest that Cu-induced inflammation is associated with conditions such as osteoarthritis, pulmonary lesions, oxidative stress, and apoptosis.[41−43] Cs toxicity has been linked to the disruption of homeostasis inflammatory mediators, neuro-inflammatory responses in rats, nephritis, and rheumatoid arthritis.[43−46]

Low levels of Cu or its deficiency can lead to chronic inflammation and oxidative stress.[47] However, not much is known about exposure to low levels of Cs. In our data, individual concentrations of Cs and Cu during pregnancy and elevated fecal calprotectin levels were not significantly associated; however, the interactive cliques of these metals (at low levels) with microbes showed strong associations with elevated fecal calprotectin. Note that prenatal exposures to metals have previously been reported to have nonlinear effects on health outcomes.[48] Therefore, such observations raise the plausibility of nonlinear synergistic interactions between Cu and Cs (only at low levels), which might affect the microbial diversity of certain bacteria. There is a lack of such interactions reported in the current literature, particularly for inflammation, but future work should investigate the mechanistic underpinnings.

Furthermore, it is well-known that metals are omnipresent elements found extensively in the environment. Upon entry into the body, they can be absorbed through the gastrointestinal tract and transported to various organs and tissues. Environmental epidemiological studies indicate that metals can influence the composition of the gut microbiome and are associated with alterations in it, affecting microbial metabolic profile, gut barrier integrity, and immune dysregulation, which are associated with gastrointestinal disorders like IBD.[49−52] Furthermore, multiple animal studies showed an association between dietary levels of Cu and inflammatory response through alteration of gut microbial compositions.[53] Therefore, exploring the interactive chemistry between metals, microbes, and their synthesis effects is essential, given the limited epidemiological studies investigating their association with child health outcomes.

A metal or metal−microbial clique signature signifies a specific affinity between metal exposures and distinct gut microbial signatures. Our prior investigations have illustrated the complex nature of these affinity clique signatures, showing unique combinations of metals and microbial abundances capable of exerting notable influences on pediatric health outcomes. For example, in our previous research using an innovative statistical method, Microbial and Chemical Exposure Analysis (MiCxA), we identified a two-taxa microbial clique signature featuring *Bifidobacterium adolescentis* and *Ruminococcus callidus* and a three-taxa clique signature that included *Prevotella clara* during late childhood, associated with lead exposure.[17] Similarly, we identified a metal−microbial clique signature characterized by high Zn levels in the second trimester, low Co levels in the third trimester, and an increased abundance of specific bacteria, such as *Bacteroides fragilis*. This precise metal−microbial clique signature was linked to elevated depression scores in school-aged children.[22] These findings emphasize the importance of these associations in disentangling how metal exposures can influence gut microbiota composition, accentuating the complex interactions between metal and microbes and thus potentially contributing to a spectrum of child health and well-being outcomes.

Strengths of our study include the use of a longitudinal birth cohort of healthy children with regular follow-up and the availability of relevant clinical data and biological samples. We used objective omics and biomarker measurement techniques and applied a state-of-the-art interpretable machine learning algorithm to identify metal−microbial clique signatures, which are novel and unbiased approaches. Our study also has limitations. Our study sample size is relatively small; however, we note a signal with a high strength of association and biological plausibility. We have conducted a cross-sectional analysis of the gut microbiome and FC due to access to stool samples at a one-time point, and there is a lack of IBD outcomes in the study participants. Although diet is an important component that affects microbial diversity,[54] we did not adjust our analyses for maternal and child diet or their dietary history due to a lack of data. Although maternal IBD status can influence child IBD and alter their gut microbial diversity,[5] we also did not adjust for maternal IBD status or calprotectin level due to the lack of such data in the present cohort. However, these data on subclinical

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features of intestinal inflammation provide important mechanistic insights. Certainly, replication in larger cohorts with a greater sample size would be an important next step toward prediction and prevention strategies in IBD. Early detection and a deeper mechanistic understanding of IBD in children reduced the risk of developing more severe conditions and associated extensive morbidity.

CONCLUSIONS

In conclusion, we demonstrate that children with specific exposure patterns to metals during pregnancy and gut microbial signatures have a higher likelihood of subclinical intestinal inflammation. Using an interpretable machine-learning technique, we provide a framework for a precision environmental health approach that identifies children with a higher susceptibility to worsened intestinal inflammation. Future research is necessary to fully understand the mechanistic insights and create an exposome-based precision health initiative.

ASSOCIATED CONTENT

Data Availability Statement

Metagenomic data are publicly available at https://www.ncbi.nlm.nih.gov/sra/PRJNA975184. All other data are available upon request to robert.wright@mssm.edu. Details on the rh-SiRF algorithm and Illustrative codes (with examples) are provided on GitHub (https://github.com/vishalmidya/MiCA-Microbial-Co-occurrence-Analysis/blob/main/MiCA-vignette.md).

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/envhealth.4c00125.

Table S1, table for prenatal metal concentrations; Figure S1, increasing and decreasing each threshold by 10 percentiles; Figure S2, associations with continuous calprotectin values; Figure S3, estimated relative risks; and Figure S4, metal−microbial clique association on test data (PDF)

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Notes

Protocols for the main PROGRESS study and its ancillary microbiome study were reviewed and approved by the Institutional Review Board at the Icahn School of Medicine at Mount Sinai (STUDY-12-00751A, STUDY-21-00242) and all

three committees (Research, Ethics in Research, and Biosafety) included at the National Institute of Public Health in Cuernavaca, Mexico.

The authors declare the following competing financial interest(s): The corresponding author confirms on behalf of all authors that there have been no involvements that might raise the question of bias in the work reported or in the conclusions, implications, or opinions stated. V.M., C.G., L.T., L.A.T.-O., J.E., M.P., J.K.G., I.P., M.M.T.-R., R.O.W., and S.E. report no conflicts of interest. M. Agrawal has consulted for Douglas Pharmaceuticals. J.J.F. is on the Scientific Advisory Board of Vedanta Biosciences. M. Arora is an employee and equity holder of Linus Biotechnology Inc., a start-up company of Mount Sinai Health System that develops tools for the detection of autism spectrum disorder and related conditions. J.-F.C. reports receiving research grants from AbbVie, Janssen Pharmaceuticals and Takeda; receiving payment for lectures from AbbVie, Amgen, Allergan Inc., Ferring Pharmaceuticals, Shire, and Takeda; receiving consulting fees from AbbVie, Amgen, Arena Pharmaceuticals, Boehringer Ingelheim, Bristol Myers Squibb, Celgene Corporation, Eli Lilly, Ferring Pharmaceuticals, Galmed Research, Glaxo Smith Kline, Geneva, Iterative Scopes, Janssen Pharmaceuticals, Kaleido Biosciences, Landos, Otsuka, Pfizer, Prometheus, Sanofi, Takeda, TiGenix; and holding stock options in Intestinal Biotech Development.

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