



## CLINICAL RESEARCH ARTICLE

# Prenatal food insecurity post Hurricane Maria is associated with decreased *Veillonella* in the infant gut

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**BACKGROUND:** Hurricane Maria struck Puerto Rico on 20 September 2017 causing catastrophic devastation. Prolonged shortage of food had been a substantial challenge to the residents after Maria. Experiencing food insecurity in utero has been associated with negative health outcomes later in life. We aim to examine whether there is any alteration in the infant gut microbiome that is associated with prenatal food insecurity.

**METHODS:** We established a cohort of infants aged 2–6 months who were exposed in utero to Hurricane Maria near San Juan, Puerto Rico and examined the gut microbiota ( $n = 29$ ) using 16S ribosomal RNA gene sequencing.

**RESULTS:** Among the enrolled infants, 30% of their mothers experienced “post-Maria poor access to food” for at least 1 month during pregnancy. The relative abundance of gut *Veillonella* spp. is significantly decreased among infants who experienced prenatal food insecurity, compared to those who did not (adjusted  $p = 0.025$ ). There is no significant difference observed by prenatal food insecurity at the microbial community level in this cohort.

**CONCLUSIONS:** Our finding indicated that infants who experienced prenatal food insecurity post hurricane harbor microbial alternations of specific bacterial taxa, which may further influence the microbial maturation and place the individual at a high-risk health trajectory.

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**IMPACT:**

- We identified that in utero exposure to food insecurity post Hurricane Maria is associated with decreased abundance of *Veillonella* in the infant gut.
- Our findings indicated that infants who experienced prenatal food insecurity post hurricane may harbor alterations of specific bacterial taxa in their gut microbiota.
- This study showed the association between prenatal adverse exposure and alterations of gut microbiome early in life in the context of an extreme event.
- This study provided insights into the mechanisms underlying prenatal adverse exposure and increased disease risks later in life.
- Our findings will potentially raise awareness of the negative impact of extreme climate events on the unborn.

**INTRODUCTION**

The deadly Category 4 tropical cyclone, Hurricane Maria, struck Puerto Rico on 20 September 2017. The whole island experienced catastrophic devastation and sustained a humanitarian crisis with an estimated death toll ranging from 2975 to 4645 and over \$90 billion in damages.<sup>1,2</sup> In the aftermath of Maria, widespread loss of electricity and communication, the collapse of the healthcare system, and the prolonged shortage of food and other critical resources, combined with delayed disaster response and aid relief, lead to substantial physical and psychological challenges to the residents of Puerto Rico.<sup>3</sup> Previous epidemiological studies have demonstrated that prenatal exposure to a devastating hurricane is associated with negative birth outcomes and increased disease

risks later in life.<sup>4–7</sup> However, due to the extremely complex mix of exposures during a hurricane crisis, the impact of each major exposure on the unborn has not been evaluated. To investigate specific exposures in the context of hurricane is a critical step to start understanding biological pathways from prenatal exposure to health outcomes.

Prenatal maternal food insecurity has been identified as a major factor that directly affects the nutrients available for the growing fetus and profoundly influences disease risks later in life.<sup>8–10</sup> With growing knowledge of the human microbiome, we have recognized that maternal diet during pregnancy plays an important role in shaping infant gut microbiota, a major stimulus for immune and metabolic function development.<sup>11,12</sup> The prenatal

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maternal diet can potentially exert its influence on the infant gut microbiota both before and after birth. In mice, prenatally, maternal nutrients and bacterial metabolites shape the infant intestinal mucosal immune cell composition, regulating the intestinal transcription pattern, which applies select pressure on the pioneer bacterial communities at birth and in early infancy.<sup>13</sup> Postnatally, maternal bacterial strains can directly transfer to the infant gut and have better ecological adaptation than those acquired from other sources.<sup>14</sup> This mother-to-infant microbial transmission has been observed for a variety of bacterial species and from multiple body sites of the mothers.<sup>14–16</sup> Additionally, food insecurity is among the top problems that are associated with stress, anxiety, or depression after a hurricane.<sup>17</sup> These effects on maternal mental health have also exhibited strong associations with alterations of the infant gut microbiome.<sup>18,19</sup> Thus, food insecurity is an important exposure for the unborn, due to its potential impact on the developing infant gut microbiota and may be the mechanism underlying prenatal disaster exposure, leading to long-term health consequences.

To this end, we conducted a study, named Hurricane as the Origin of Later Alterations in microbiome (HOLA), with infants who were exposed in utero to Hurricane Maria in the San Juan greater metropolitan area. We investigated their major prenatal exposures due to Hurricane Maria and evaluated the gut microbiome by prenatal maternal food security state.

## METHODS

Subject recruitment, sample collection, and DNA extraction  
HOLA study was reviewed and approved by the Institutional Review Boards of San Juan City Hospital (ID: EPDC-Microbiome) and deemed exempt by Washington University Human Research Protection Office as all data was de-identified. All parents signed a written informed consent for infant–mother pair participation in the study. Between April and August 2018, we recruited infants through San Juan City Hospital Research Unit. Study inclusion criteria were (1) child's mother resided in Puerto Rico throughout the pregnancy; (2) aged more than 2 months and <6 months; (3) born at term via vaginal delivery. The exclusion criteria include (1) delivery by cesarean section; (2) premature birth (defined as <37 weeks of gestational age at the time of birth); (3) admission to Neonatal Intensive Care Unit; (4) acute illness at the time of acquiring the samples; (5) airway or pulmonary malformations; (6) identified chromosomal or genetic abnormalities.

The infants' fresh stool samples were collected at the clinic or during a home visit by a trained health professional, taken directly from the diaper liner using a sterile swab. The swab was then immediately placed into the DNA/RNA Shield collection solution (Zymo Research, Irvine, CA), and shipped to Washington University for bacterial DNA extraction. The DNA/RNA Shield collection tube contains DNA and RNA stabilization solution, which preserves the integrity of nucleic acids of the samples. Therefore, the storage and transportation of collection tubes were at ambient temperatures. Thirty infants were recruited and 29 stool samples were used for final analysis since one subject was under antibiotic treatment when the stool sample was collected.

At the time of sample collection, we conducted a questionnaire-based interview in Spanish with the mother of each subject. The questionnaire was based on various sources, including personal experience with disaster of some of the members of the research teams, particularly those who lived or were actively involved in disaster relief immediately after the Hurricane. The questions assessing hurricane exposures are listed in Supplementary File 2. We additionally administered the Edinburgh Postnatal Depression Scale (EPDS), a standard psychological instrument for detection of depressive symptoms.<sup>20</sup> Two researchers independently extracted data from the questionnaire and calculated EPDS score. Any discrepancy in the data input was carefully discussed to resolve.

DNA extraction, PCR amplification, and sequencing of bacterial 16S ribosomal RNA genes

Total genomic DNA was extracted from the swab collection using Zymo Fecal/Soil Microbe Miniprep Kit (Zymo Research, Irvine, CA). Fourteen PCR amplicons, representing all nine 16S variable regions, were produced using the Fluidigm Access Array System. Five nanograms per microliter of DNA were input into each reaction. The reaction mix consisted of 1X High Fidelity FastStart Reaction Buffer without MgCl<sub>2</sub> (Roche), 4.5 nM MgCl<sub>2</sub> (Roche), 5% dimethyl sulfoxide (Roche), 200 μM PCR Grade Nucleotide Mix (Roche), 0.05 U/μL of 5 U/μL FastStart High Fidelity Enzyme Blend (Roche), 1X Access Array Loading Reagent (Fluidigm), 1 μL DNA, and water. Two hundred nanomoles each of the forward and reverse primers and 1X Access Array Loading Reagent were added to the reaction mix. PCR amplification was performed on the BioMark HD system from Fluidigm. The reaction products were harvested and indexed using unique 10 bp sequences with 14 rounds of PCR to incorporate each index sequence. All samples were pooled into 48 sample libraries and cleaned using bead purification. Libraries were sequenced using an Illumina MiSeq sequencer (2 × 150 base pair kit). Amplification and sequencing was performed at the Genome Technology Access Center at Washington University.

16S ribosomal RNA gene sequence processing

Of the 14 PCR amplicons sequenced, reads from one amplicon, which covers the 16S V4 variable regions, were first examined using the QIIME pipeline. Reads from the other 13 amplicons were either discarded or used for the MVRISION taxonomic analysis described below. The paired end reads were joined into single sequences using fastq-join with the default parameters where the minimum length of the overlap is 6 and the maximum hamming distance considered is 8% of the overlap length. Only the joined V4 reads were used for analysis using the QIIME pipeline version 1.9.0.<sup>21</sup> Open-reference operational taxonomic units (OTUs) were called using the Greengenes May 2013 release as the reference database.<sup>22</sup> Reads were clustered into OTUs by QIIME using UCLUST at a threshold of 97% similarity.<sup>23</sup> Representative sequences for each OTU were classified taxonomically with the UCLUST consensus taxonomy assigner in QIIME using a sequence similarity of 0.9. The resultant OTU table was rarefied to the lowest individual total read count of 5600.

To optimize taxonomic assignment, we then used the MVRISION pipeline, which uses sequencing reads from 7 of the 14 amplicons, to produce a list of microbial species along with the number of reads assigned to each species for each sample.<sup>24</sup> MVRISION was run with default parameters, except for the novoalign -tval parameter of 180. The data was run on a filtered version of the SILVA 16S database.<sup>25</sup> MVRISION produces a taxon read count table where the counts are the number of reads classified for each species averaged across all variable regions. This taxon read count table was rarefied to a depth of 2390 reads.

Statistical analysis

α-Diversity (e.g., Shannon index) and β-diversity (e.g., weighted UniFrac distance) calculations were performed from rarefied reads in the V4 region. ANOSIM (ANALYSIS of SIMilarities) comparisons of β-diversity distance matrices by prenatal malnutrition status were performed in QIIME. A random forest machine-learning model was used to rank feature importance of the bacteria genera in predicting prenatal exposure to food insecurity. The prediction performance was evaluated using the relative abundance matrices at genus level. We applied Leave-One-Out as the dataset splitting method in which each sample is used once as a test set (singleton), while the remaining samples for the training set. Default parameters were chosen on the Scikit-learn python package v.0.19.1.<sup>26</sup> The algorithm was repeated 999 times to generate a ranked list in the order of feature importance. R

package DESeq2 was used to detect differential bacteria genera using a generalized linear regression model with a logarithmic link and following a negative binomial distribution.<sup>27</sup> Resulting *p* values were adjusted for multiple testing according to the Benjamini–Hochberg procedure. We used R v.3.4.2 and Python3 for statistical analyses and “ggplot2” for visualization.<sup>28</sup>  $\alpha$  Values < 0.05 were considered statistically significant.

## RESULTS

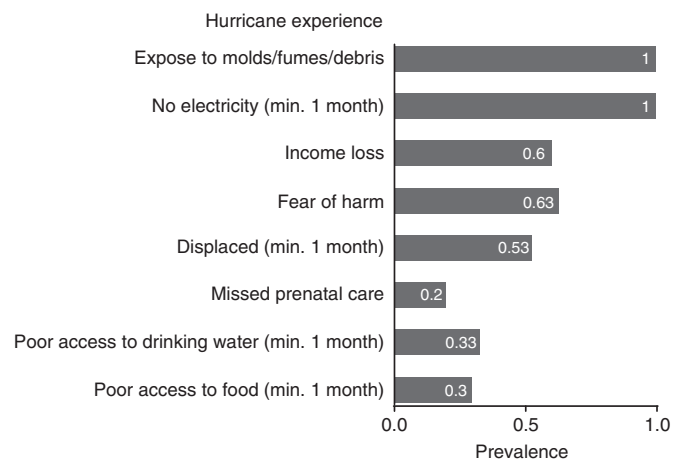
Prevalence of prenatal maternal food insecurity in HOLA and the gut microbiome at the community level

We enrolled 30 infants aged 2 to 6 months in the HOLA study. All of our subjects resided in the same metropolitan area as defined by the US Census Bureau. The municipalities included are interconnected by main highways and on an average day can be reached from San Juan proper within an hour's drive time. In the aftermath of Hurricane Maria, limited access to fuel, 100% power outage, and loss of local crops affected all areas equally. The prevalence of major hurricane consequences for their mothers during pregnancy is summarized in Fig. 1. All the participants' mothers experienced at least 1 month of power outage due to the hurricane and were exposed to molds, fumes, or debris. Thirty percent of the mothers experienced “poor access to food due to Hurricane Maria” for at least 1 month. One participant was under antibiotic treatment for diarrhea when we collected stool samples, so we excluded this subject for microbiome analysis. The demographics of the 29 subjects were described in Table 1. We compared infants whose mothers answered that the household had poor access to “food” due to hurricanes Maria and Irma (occurred 2 weeks prior to Maria) during gestation ( $n = 8$ ) and infants whose mothers answered that the household did not have poor access to food during gestation ( $n = 21$ ).

In QIIME, from a total of 226,771 reads, we observed 263 OTUs. The OTU table is in Supplementary File 3. The most abundant phyla in the gut microbial community were Firmicutes (37%), Actinobacteria (27%), Bacteroidetes (20%), and Proteobacteria (15%). To evaluate general features of the infant microbiome, we compared the  $\alpha$ -diversity (Shannon's index) by infant feeding types and age, influential factors on the infant gut microbial community. As shown in Fig. 2a, feeding type was significantly associated with the gut microbial community composition, with exclusive breast milk feeding resulting in significantly lower  $\alpha$ -diversity compared to exclusive formula feeding ( $p = 0.02$ ), which is consistent with previous studies.<sup>29</sup> In Fig. 2b, the overall  $\alpha$ -diversity shows an increasing trend with infant age, but likely since we captured a narrow age window (2–6 months old), the increase was not significant. In Supplementary Fig. S1A–C (online), the principal coordinate analysis of weighted UniFrac distance shaded for the relative abundance of three major families, Bifidobacteriaceae, Bacteroidaceae, and Enterobacteriaceae, highlights three distinct clusters. Supplemental Figure S1D–F (online) further shows that the composition of the three major bacterial families is influenced by feeding type with Bifidobacteriaceae significantly correlated with breastfeeding vs. formula feeding ( $p = 0.03$ ). Notably, there is no significant difference in the  $\alpha$ - or  $\beta$ -diversity measured by Shannon's index (Wilcoxon,  $p = 0.83$ ) or weighted UniFrac distances (ANOSIM,  $p = 0.69$ ) by prenatal food security state (Fig. 2c, d).

Decreased relative abundance of *Veillonella* spp. is associated with prenatal maternal food insecurity

The relative abundance of most prevalent genera (present in >20% of subjects, 38 in total), grouped by prenatal maternal food security state, is shown in Fig. 3. To evaluate the potential impact of prenatal food insecurity on the infant microbiome, we conducted a random forest machine-learning model to rank the 38 most prevalent genera by their feature importance on



**Fig. 1** Adverse experience during the aftermath of Hurricane Maria. Prevalence of self-reported experience due to Hurricane Maria among the mothers of 30 participating infants.

**Table 1.** Characterization of the microbiome study cohort<sup>a</sup>.

	Maternal access to food		<i>p</i> Value
	Poor ( <i>n</i> = 8)	Normal ( <i>n</i> = 21)	
Ethnicity (Latino)	8 (100%)	21 (100%)	
Vaginally delivered	8 (100%)	21 (100%)	
Gestational age (weeks)	39 ± 0.84	39 ± 1.07	0.73
Trimester on the day of Hurricane Maria			0.46
1st	3 (37.5%)	5 (23.8%)	
2nd <sup>b</sup>	5 (62.5%)	16 (76.2%)	
Gender (male)	2 (25%)	8 (38.1%)	0.51
Infant age at sample collection (weeks)	18 ± 5.9	15 ± 5.2	0.18
Feeding type			0.43
Exclusive breast milk	2 (25.0%)	6 (28.6%)	
Mixed	5 (62.5%)	8 (38.1%)	
Exclusive formula	1 (12.5%)	7 (33.3%)	
Maternal EPDS score	6.75 ± 7.09	4.90 ± 3.78	0.50

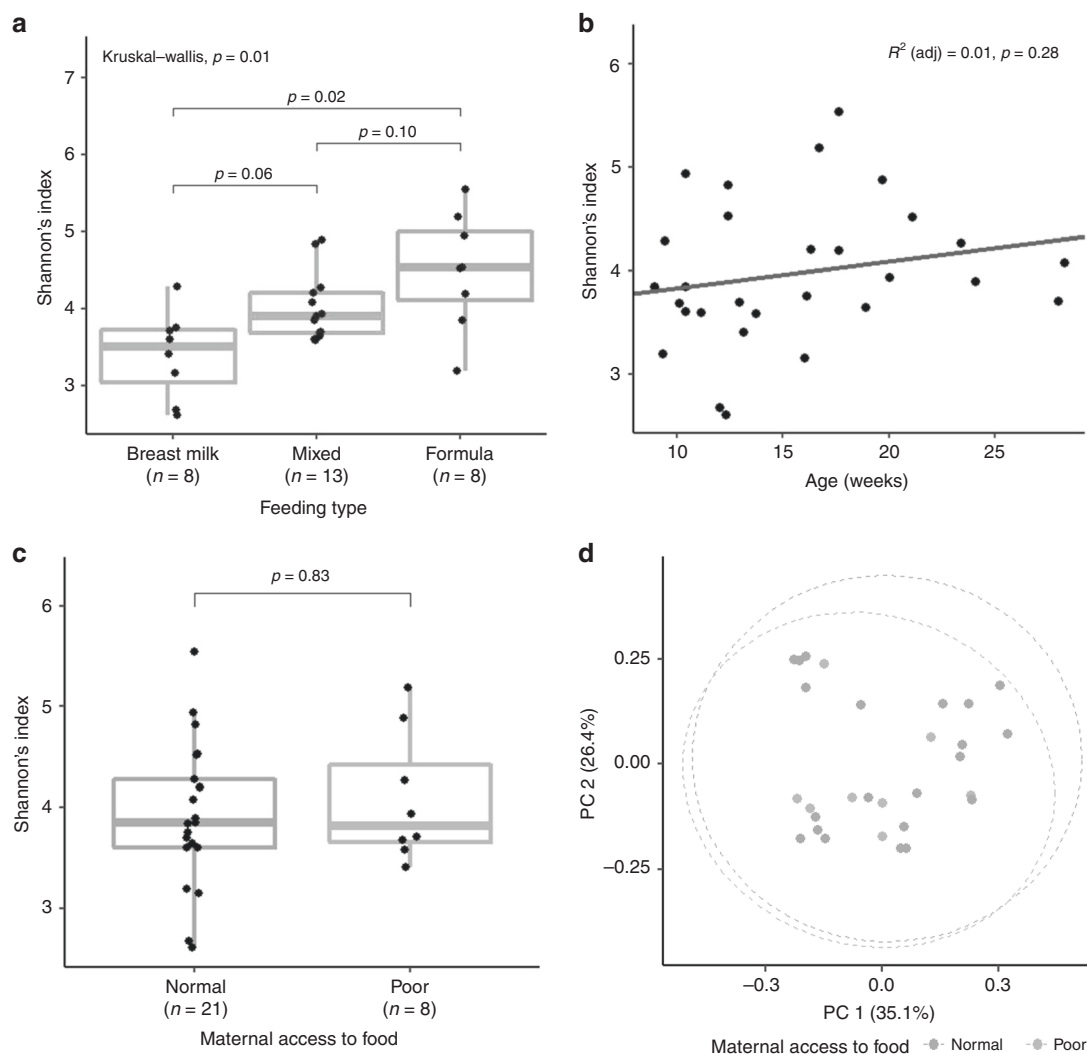
Statistical significance was determined by *t* test or  $\chi^2$  test. Values are presented as number (percentage) or mean ± SD.

SD standard deviation, EPDS The Edinburgh Postnatal Depression Scale.

<sup>a</sup>Totally 29 infants were included in the gut microbiome study. One participant was under antibiotic treatment for diarrhea when we collected stool samples, so we excluded this subject for microbiome analysis.

<sup>b</sup>One of the subjects was 30-week pregnant.

predicting the prenatal food security status. With the cross-validation accuracy of 0.724 in the model, genus *Veillonella* is ranked as the most important feature with the importance score of 0.13, nearly doubled the importance score of the second-best feature, genus *Erwinia* with the importance score of 0.07 as shown in Fig. 4a and Supplementary Table S1 (online). We then applied a generalized linear regression model using DESeq2, including all 206 genera to identify bacterial genera with significant differential abundance by prenatal food security. After adjusting for multiple comparisons, *Veillonella* is significantly decreased in infants who experienced food insecurity during gestation, compared to infants who did not (means of relative abundance: 2.3% vs. 7.6%, adjusted  $p = 0.025$ , Fig. 4b and Supplementary Table S2 (online)).



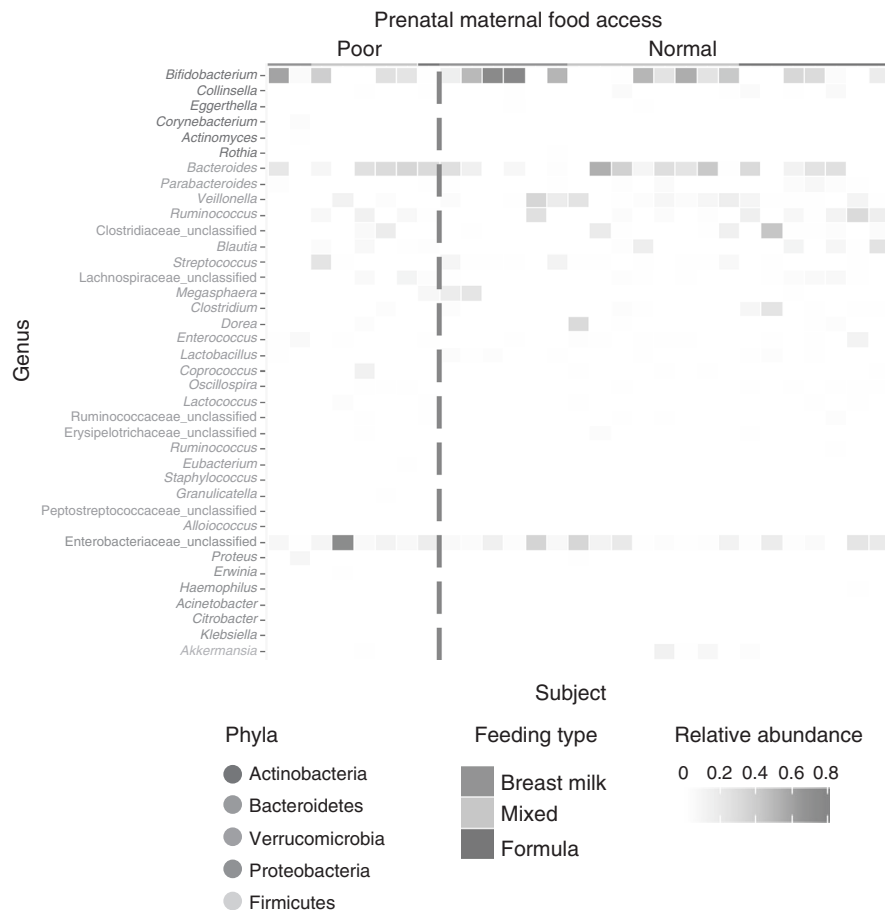
**Fig. 2** The gut microbial community evaluated by  $\alpha$ - and  $\beta$ -diversity. **a** Shannon's index by feeding types. **b** Shannon's index by infant age. The blue line represents the best-fit line in the linear regression model. **c** Shannon's index by prenatal maternal food security status. Statistical significance in **a–c** was determined using Kruskal–Wallis test, Wilcoxon's rank-sum test, or linear regression model. **d** Principal Coordinate Analysis (PCoA) plot based on the weighted UniFrac distance, colored by prenatal maternal food security status (ANOSIM,  $p = 0.69$ ). PC1, principal coordinate axis 1; PC2, principal coordinate axis 2.

We then performed MVRSION, a novel 16S sequencing and analysis pipeline to validate this observed association in QIIME. We compared the resulting relative abundance of *Veillonella* spp. between the traditional QIIME pipeline and MVRSION pipeline. As shown in Fig. 3D, the abundance of *Veillonella* spp. are consistent from the two methods ( $R^2 = 0.82$ ,  $p = 7.34 \times 10^{-12}$ ). MVRSION results show the same significant difference between food insecurity and security groups regarding the relative abundance of *Veillonella* (Supplementary Fig. S2A (online)). Furthermore, MVRSION identified seven species in genus *Veillonella* in our samples, including *V. parvula*, *V. dispar*, *V. ratti*, *V. magna*, *V. cricetid*, *V. atypica*, and *V. tobetsuensis*. As shown in Supplementary Fig. S2B (online), with an increase in the relative abundance of *Veillonella* spp, more species were present, regardless of the food security status, which indicated a correlation between the relative abundance and species diversity of *Veillonella* spp. ( $R^2 = 0.55$ ,  $p = 2.19 \times 10^{-6}$ ).

The identified association with *Veillonella* spp. is not confounded by feeding types, or infant age. Also, this association is not with hurricane-related drinking water insecurity or displacement. To investigate whether feeding type is confounding the association of *Veillonella* spp. with prenatal food insecurity, we

divided the subjects into breast milk or mixed fed group and exclusively formula fed group. As shown in Fig. 5a, regardless of feeding type, the trends are similar: infants experiencing prenatal food insecurity have less abundant *Veillonella* spp. when compared to the infants whose mother did not experience food insecurity. In the breast milk/mixed group, there is a significant decrease of *Veillonella* spp. in the prenatal food insecure group ( $p = 0.04$ ). With smaller numbers of subjects in the formula group, we did not observe significant difference, but can still see the same trend. Infant age is not correlated with *Veillonella* spp. ( $p = 0.64$ , data not shown) and the age distributions are not different between these two groups ( $p = 0.18$ , Table 1). However, the two groups displayed opposite trends regarding the relative abundance of *Veillonella* with age. As shown in Fig. 5b, relative abundance of *Veillonella* spp. increases with age in the control group. However, the converse is true in the prenatal food insecurity group.

We then examined the association of *Veillonella* spp. with other top hurricane-related stressors, including drinking water insecurity and displacement.<sup>17,30</sup> As shown in Supplementary Fig. 3A (online), the relative abundance of *Veillonella* spp. is not associated with prenatal drinking water security. The mean relative



**Fig. 3** The relative abundance of genera, grouped by food access state, the infant feeding type, and the infant age. Each column represents one infant. The most prevalent genera (present in at >20% subjects) are included, sorted vertically by phylum and total abundance of all subjects.

abundance of *Veillonella* in drinking water secure group vs. drinking water insecure group is 6.04% vs. 6.34% ( $p = 0.68$ ). Similarly, in Supplementary Fig. 3B (online), the relative abundance of *Veillonella* spp. is not associated with displacement. The mean relative abundance of not-displaced group vs. displaced group is 5.27% vs. 9.52% ( $p = 0.29$ ). These results did not show association of *Veillonella* abundance with two other hurricane stressors, prenatal drinking water insecurity or displacement.

## DISCUSSION

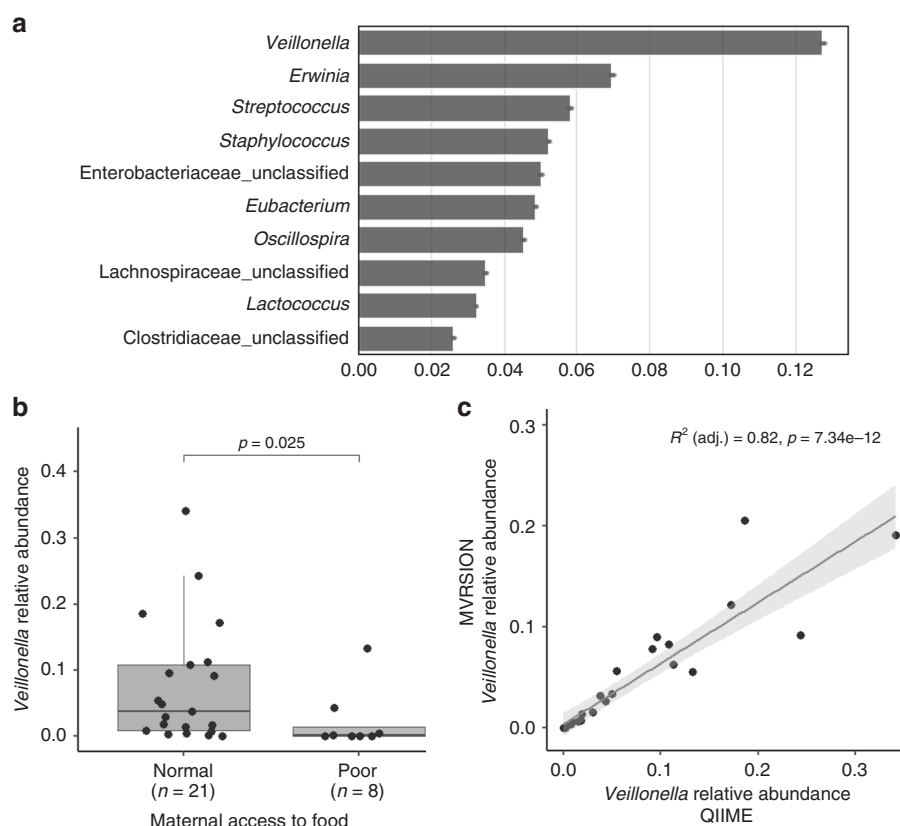
This is the first human study to examine the impact of maternal prenatal food insecurity after a devastating hurricane on one specific aspect of infant outcomes, the early-in-life gut microbiome. We identified that the relative abundance and species diversity of gut *Veillonella* spp. is significantly decreased among infants who had prenatal maternal food insecurity, compared to those who did not. Our findings indicate that infants who experienced prenatal food insecurity post hurricane may harbor alterations of specific bacterial taxa in their gut microbiota, which may further influence the individual's microbial maturation, immune development, and long-term health. There are two possible routes through which maternal food insecurity affects offspring gut microbiota: (1) the maternal nutritional availability is associated with an altered in utero environment that influences fetal programming, resulting in changes to the fetal gut niche that then imposes selective pressures on microbiota early-in-life; (2) an altered maternal microbiota due to events surrounding

hurricane Maria can be vertically transmitted to the offspring prenatally and/or postnatally. Further studies are needed to investigate these two potential routes.

The microbial alteration among prenatal food insecure infants may be attributable to nutrition deficiency as well as maternal mental distress related to food insecurity. Previous studies on Hurricane Katrina have found that lack of basic necessities, such as food, drinking water, or displacement is strongly associated with post-traumatic stress, anxiety, and depression.<sup>17</sup> While mothers in the prenatal food insecure and secure groups had no significant difference in terms of postpartum depression, according to the EPDS tests shown in Table 1, we lacked evaluation of the maternal distress during gestation to delineate the potential impact of food insecurity on the prenatal stress. We examined whether *Veillonella* was associated with other hurricane stressors. We found that there was no significant difference in the relative abundance of *Veillonella* spp. by drinking water security, or displacement experience. However, considering all the mothers experienced a complex mix of chronic stress during gestation, their distress level may not be well reflected by a certain adverse experience in the aftermath of Maria. Therefore, we could not tease out the potentially critical role maternal distress may play on the association.

As shown by our analyses of  $\alpha$ - and  $\beta$ -diversity, the microbial community structure of our cohort was influenced by feeding types, which has also been seen in previous studies of other infant cohorts.<sup>29</sup> This indicated that prenatal food insecurity may not disturb the microbial community structure of the gut of infants





**Fig. 4** *Veillonella* spp. is significantly associated with prenatal food insecurity. **a** The top 10 most important genera in predicting maternal prenatal food security status. The most prevalent 38 genera (present in at >20% subjects, list in **a**) were included in the random Forest model (999 replicates). The x-axis shows the importance of the genus to the accuracy. The error bar represents the standard error. **b** The relative abundance of *Veillonella* spp. identified in QIIME by prenatal maternal food security status. Statistical significance was determined using Wilcoxon's rank-sum test. **c** Correlation of *Veillonella* spp. relative abundance calculated by QIIME and MVRSION in the linear regression model. The blue line represents the best-fit line and the shaded gray area represents the 95% confidence interval.

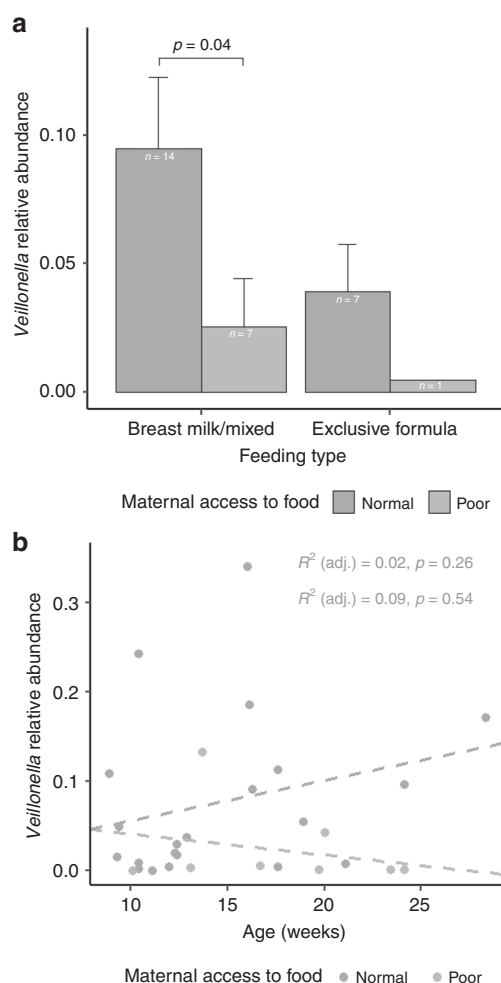
aged 2 to 6 months. However, a previous study has revealed that *Veillonella* spp. is a dominant taxa in defining ecological structures at age 6 months.<sup>31</sup> Therefore, it is possible that the decrease of *Veillonella* spp. early in life has the potential to initiate structural disturbance, which would not be seen until later in life. Follow-up studies are needed to assess long-term effects of prenatal maternal food insecurity on the offspring gut microbiome at both community and taxa levels.

*Veillonella* spp. are Gram-negative anaerobic flora commonly present in multiple body sites, such as mouth, gastrointestinal tract, vagina, and breast milk.<sup>32–35</sup> *Veillonella* spp. is one of the major producers of short-chain fatty acid (SCFA).<sup>36,37</sup> Low levels of SCFA have been associated with diseases in which inflammatory processes are involved.<sup>38,39</sup> In the Canadian Healthy Infant Longitudinal Development study, *Veillonella* was among the four genera which were significantly reduced in the early infancy among children at high risk of asthma.<sup>39</sup>

Several limitations should be acknowledged in the current study. First, because the cohort size is relatively small, we may not have enough power to detect more subtle changes in the prenatal malnutrition-associated bacterial taxa. Potentially larger effects of prenatal food insecurity on the infant gut microbiome may exist. Therefore, repeated and longitudinal evaluations of the infant gut microbiome in a large-scale cohort post disaster will be necessary to confirm and extend our findings and further examine the influence of prenatal food insecurity after a disaster. Second, although we evaluated potential confounding factors, some important information may still be missing in the

current study, such as socioeconomic status, sanitation, and pathogen burden in the residing area. These factors can affect food accessibility after Hurricane Maria or directly disturb the gut microbiota. Third, our questionnaire provided us with objective and categorical information to define the state of food access in the household due to Hurricane Maria. Detailed questions regarding the access to different food categories and the major reasons causing food insecurity in a similar cohort would provide a more broad and in-depth view of the severe and prolonged food insecurity problem post Hurricane Maria. Fourth, although our findings focused on the food insecurity issue in the aftermath of Hurricane Maria, the disaster caused widespread destruction and devastating losses to the whole island. More research is warranted to investigate the link between in utero exposure to this deadly disaster and biological markers related to the health outcomes.

In summary, HOLA is the first study to examine the influence of prenatal maternal food insecurity after a devastating hurricane on the offspring microbiota. We identified a significant association between limited prenatal access to nourishment and decrease in infant *Veillonella* spp. While not causal, our findings begin to provide evidence of an underlying mechanism linking in utero exposure to a disaster and increased disease risk later in life. Future human cohort and animal studies will help to validate our findings and determine the mechanisms by which lack of nourishment during pregnancy in the context of a substantial hurricane influences the relative abundance of certain gut microbial taxa in the offspring in early infancy.



**Fig. 5 Subgroup analysis for the association between *Veillonella* spp. and prenatal food insecurity.** **a** The relative abundance of *Veillonella* spp. by feeding types and prenatal maternal food security status. Values are means  $\pm$  SEM. Statistical significance was determined using Wilcoxon's rank-sum test. **b** The relative abundance of *Veillonella* spp. vs. age by prenatal maternal food security status. Statistical significance was determined using linear model. The lines are the best-fit line in the corresponding linear regression model by maternal food security status, respectively.

## DATA AVAILABILITY

The 16S ribosomal RNA sequencing files have been deposited into the European Nucleotide Archive (ENA). The accession code is PRJEB32915.

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## AUTHOR CONTRIBUTIONS

L.W., N.R., and D.A. conceived and coordinate the study. K.G., N.R., and M.A. recruited subjects. D.A., and A.C. extracted data from questionnaire. A.C. and L.W. processed

samples for sequencing. A.S. and L.W. performed sequencing data analysis. B.W., L.S., and L.H. provided advice on the study design and analysis. L.W. wrote the manuscript. B.W., L.S., L.H., A.S., and D.A. provided comments and edits on the manuscript. All authors read and approved the manuscript.

## ADDITIONAL INFORMATION

The online version of this article (<https://doi.org/10.1038/s41390-020-0839-0>) contains supplementary material, which is available to authorized users.

**Competing interests:** The authors declare no competing interests.

**Patient consent:** All the parents of the participating infants signed a written informed consent.

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