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The role of gut microbiota in fetal methylmercury exposure: Insights from a pilot study

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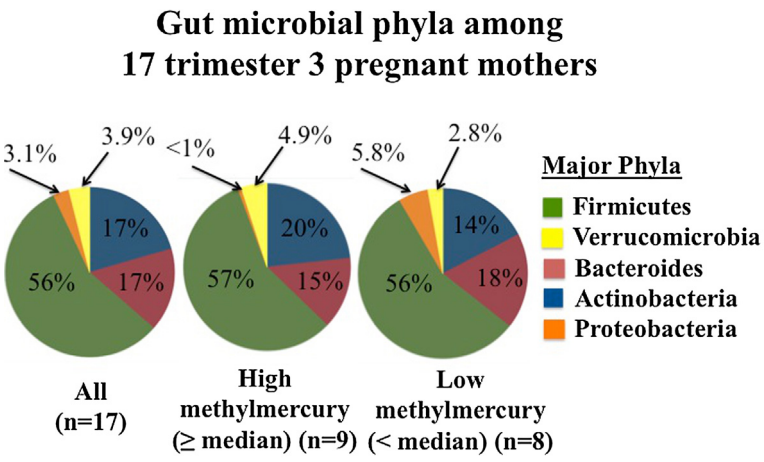
HIGHLIGHTS

- Mercury methylation/demethylation did not likely contribute to stool methylmercury.
- Maternal stool mercury was not correlated with maternal hair or cord blood mercury.
- Seventeen bacterial genera were correlated with mercury in maternal stool or hair.
- For these correlations, gut microbiota functions (utilized or affected) are unknown.

GRAPHICAL ABSTRACT



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ABSTRACT

Purpose: The mechanisms by which gut microbiota contribute to methylmercury metabolism remain unclear. Among a cohort of pregnant mothers, the objectives of our pilot study were to determine (1) associations between gut microbiota and mercury concentrations in biomarkers (stool, hair and cord blood) and (2) the contributions of gut microbial mercury methylation/demethylation to stool methylmercury.

Methods: Pregnant women (36–39 weeks gestation, $n = 17$) donated hair and stool specimens, and cord blood was collected for a subset ($n = 7$). The diversity of gut microbiota was determined using 16S rRNA gene profiling ($n = 17$). For 6 stool samples with highest/lowest methylmercury concentrations,

Abbreviations: BDL, below detection level; BMI, body mass index; CH_2Cl_2, dichloromethane; CMMR, Alkek Center for Metagenomics and Microbiome Research; CVAFS, cold vapor atomic fluorescence spectrometry; DDI-H_2O, double-distilled water; FDR, False Discovery Rate; GC, gas chromatography; Hg, mercury; HMP, Human Microbiome Project; IHg, inorganic mercury(II); IOM, Institute of Medicine of the National Academies; KEGG, Kyoto Encyclopedia of Genes and Genomes; MeHg, methylmercury; NA, not applicable; NRC, National Research Council; ORNL, Oak Ridge National Laboratory; OTU, Operational Taxonomic Units; PCoA, Principal Coordinates Analysis; QA/QC, Quality Assurance/Quality Control; QIIME, Quantitative Insights Into Microbial Ecology; THg, total mercury; USEPA, U.S. Environmental Protection Agency; WGS, whole genome shotgun.

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metagenomic whole genome shotgun sequencing was employed to search for the mercury methylation gene (*hgcA*), and two *mer* operon genes involved in methylmercury detoxification (*merA* and *merB*).
Results: Seventeen bacterial genera were significantly correlated (increasing or decreasing) with stool methylmercury, stool inorganic mercury, or hair total mercury; however, aside from one genus, there was no overlap between biomarkers. There were no definitive matches for *hgcA* or *merB*, while *merA* was detected at low concentrations in all six samples.
Major conclusions: Proportional differences in stool methylmercury were not likely attributed to gut microbiota through methylation/demethylation. Gut microbiota potentially altered methylmercury metabolism using indirect pathways.

1. Introduction

Microbes modulate the toxicity of mercury (Hg) through methylation of less toxic inorganic Hg(II) (IHg) and demethylation (i.e., detoxification) of methylmercury (MeHg) (Barkay et al., 2003; Gilmour et al., 2013; Parks et al., 2013; Smith et al., 2015). The distal gastrointestinal tract is one of the most densely populated ecosystems, with greater than 10^{11}–10^{12} organisms per mL of luminal content. Thus the gut is potentially an important reservoir for Hg cycling and MeHg metabolism.

Several animal and human studies confirmed gut microbiota modulate the enterohepatic cycling of MeHg. Co-consumption of high-fiber foods, including wheat and fruit, were associated with lower absorption of MeHg into tissues (Passos et al., 2007; Rowland et al., 1984, 1986), presumably due to higher elimination of MeHg. Treatment of animals with antibiotics reduced decomposition of MeHg in the large intestine compared to controls (Seko et al., 1981), and increased the half-time of MeHg elimination (Rowland et al., 1984), implicating gut microbes in MeHg metabolism. Microbial MeHg detoxification involves MerA, the mercuric reductase, and MerB, the organomercurial lyase (Barkay et al., 2003). Both genes encoding these proteins were recovered from human and non-human primate feces, verifying decomposition of Hg species by gut microbiota potentially occurred (Liebert et al., 1997). Tanzanian pregnant women who consumed probiotics daily (i.e., yogurt) had significantly lower blood Hg concentrations compared to controls, matched by age, nutritional status and fish intake (Bisanz et al., 2014). Methylation of IHg by gut microbes is also possible; to date, one commensal methanogen (*Methanomassiliicoccus luminyensis*) isolated from human feces (Dridi et al., 2012) contained the gene cluster (*hgcA* and *hgcB*) required for IHg methylation (Parks et al., 2013). However, the capacity of intestinal bacteria to methylate Hg was observed by some researchers (Rowland et al., 1975), but not others (Zhou et al., 2011). The mechanisms by which gut microbes contribute to MeHg cycling in the human body remain unclear.

This pilot study involves trimester 3 pregnant women because the fetus is the most vulnerable population to the deleterious effects due to MeHg exposure (National Research Council (NRC), 2000), and trimester 3 coincides with the period when brain growth is most rapid. Pregnancy is also associated with changes in gut microbiota composition (Koren et al., 2012; Santacruz et al., 2010), which may alter MeHg metabolism, and hence fetal MeHg exposure. The following hypotheses were investigated: (1) gut microbiota alter the speciation and toxicity of Hg through Hg(II) methylation and/or MeHg demethylation, and (2) gut microbiota affect MeHg bioavailability to the developing fetus by altering Hg speciation.

2. Materials and methods

2.1. Recruitment

Between November and December 2013, pregnant women from the Greenville Health System (Greenville, South Carolina, USA) were invited to participate, who were 36–39 weeks pregnant, at least 18 years of age, and in good general health. Nineteen mothers provided informed consent, including 17 mothers who donated both hair and stool samples (89%). Cord blood samples were obtained for a subset ($n = 7$, 37%). Trimester 1 (8–12 weeks gestation) body mass index (BMI) (kg/m^2), weight change during pregnancy, ethnicity, and antibiotic treatment were determined from the medical record (Table 1). Adherence to Institute of Medicine (IOM) of the National Academies Guidelines for healthy weight gain during pregnancy (Institute of Medicine of the National Academies (IOM), 2009) was determined from trimester 1 BMI and weight change at recruitment. Protocols were approved by the Institutional Review Boards at Greenville Health System and the University of South Carolina.

2.2. Biomarker collection and preservation

A hair sample was collected from the occipital region, which was tied with a string and stored in a plastic bag at room temperature. Mothers were given a stool collection kit including sterile collection containers (Thermo Fisher 02-544-208) and detailed instructions adapted from the Human Microbiome Project protocols (Human Microbiome Project (HMP), 2010). Participants shipped stool samples overnight to the University of South Carolina. Upon receipt, stool samples were aliquoted using sterile microspatulas (Corning 3012) into 2 mL sterile cryovials (Thermo Fisher 50-476-502), and then a separate sample was aliquoted using acid-washed utensils

Table 1
Demographic data for 17 participants.

	Average ± 1 SD (range) or *N* (%)
Gestation (weeks)	37 ± 0.94 (36–39)
Trimester 1 BMI (kg/m^2)	29 ± 6.7 (16–40)
Weight gain (kg)	14 ± 7.8 (1.4–33)
Ethnicity	
White	7 (41)
Black	7 (41)
Hispanic	3 (18)
Weight class	
Underweight	1 (5.9)
Normal	3 (18)
Overweight	5 (29)
Obese	8 (47)
IOM Guidelines	
Below	2 (12)
Within	6 (35)
Above	9 (53)
Antibiotic treatment previous 3 months	
No	10 (59)
Yes	6 (35)
Uncertain	1 (5.9)

Institute of Medicine (IOM), trimester 1 body mass index (BMI).