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Genetic modification of the effect of maternal household air pollution exposure on birth weight in Guatemalan newborns

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Abstract

Low birth weight is associated with exposure to air pollution during pregnancy. The purpose of this study was to evaluate whether null polymorphisms of Glutathione S-transferases (GSTs), specifically GSTM1 and GSTT1 genes in infants or mothers, modifies the association between high exposures to household air pollution (HAP) from cooking fires and birth weight. Pregnant women in rural Guatemala were randomized to receive a chimney stove or continue to use open fires for cooking. Newborns were measured within 48 hours of birth. 132 mother-infant pairs provided infant genotypes (n=130) and/or maternal genotypes (n=116). Maternal null GSTM1 was associated with a 144 gram (95% CI: -291, 1) and combined maternal/infant null GSTT1 was associated with a 155 gram (95% CI -303, -8) decrease in birth weight. Although there was a trend toward higher birth weights with increasing number of expressed GST genes, the effect modification by chimney stove use was not demonstrated.

Keywords

Genetic susceptibility; Glutathione S-transferase; low birth weight; gene-environment interaction; RESPIRE/CRECER studies; woodsmoke

1. Introduction

Low birth weight, a significant cause of infant morbidity and mortality, is associated with maternal exposure to air pollution during pregnancy. Evidence of this association is drawn

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from at least two dozen studies in developed countries [1-3] where air pollutant concentrations are relatively low and low birth weight (LBW, newborn weight < 2500 grams) is relatively rare. More than 95% of all LBW infants are born in low income countries [4]. In these countries, approximately 80% of rural households are exposed to a major source of air pollution, smoke inside the home generated by cooking and heating with solid fuels (e.g. wood)[5].

Under these conditions, pregnant women are highly exposed to toxic by-products of combustion from cooking fires, and these maternal exposures impact newborn birth weight. A systematic review with a meta-analysis of five studies that examined the relationship between maternal exposure to solid fuels and birth weight [6-10] found a 38% increased risk of LBW (OR 1.38, 95% CI, 1.25, 1.52) among those exposed to smoke from solid cooking fuel [11]. Two of these studies were conducted in Guatemala [8, 10], where 57% of all households [12] and 85% of rural household use wood fuel [5].

Not all pregnant women exposed to air pollution have low birth weight infants. The reason for this may be differential genetic susceptibility. Maternal genetic polymorphisms were found to modify the associations between birth weight and tobacco smoke [13-23] and birth weight and outdoor air pollution exposures [22, 24]; several studies even found independent gene effects on birth outcomes [25-27]. Two isoforms of the Glutathione S-transferase super gene family, GSTM1 and GSTT1, have been proposed as candidate genes for susceptibility to inhaled oxidants from air pollutants. The modifying effect that maternal and infant genotypes of GSTM1 and GSTT1 have on exposures to solid fuel smoke and the resulting impact on newborn birth weight are unknown.

Three major air pollutants from solid fuels burned in inefficient, poorly ventilated cookstoves are particulate matter (PM), carbon monoxide (CO) and polycyclic aromatic hydrocarbons (PAHs) [28, 29]. The toxicity of PM, a composite mixture of liquid drops and solid particles suspended in air, may be related to the physical and chemical properties of the particles, such as particle size and surface composition [30, 31]. For health effects, PM is most commonly classified by the mean aerodynamic diameter of the particle, with smaller particles exerting larger effects. In Guatemala, 48-hour kitchen concentrations of PM_{2.5} (PM with aerodynamic diameter < 2.5 µm) averaged 636 µg/m³ (Standard Deviation (SD): 402 µg/m³, n=50) in homes using open fires for cooking and 69 µg/m³ (SD: 89 µg/m³, n=49) in homes using well-maintained chimney stoves [32]. However, even the kitchen concentrations where chimney stoves were used exceed the World Health Organization Air Quality Guidelines of 25 µg/m³ for PM_{2.5} averaged over a 24-hour period [33].

Carbon monoxide (CO) is a product of incomplete combustion released when organic solid fuels, such as wood, are burned with insufficient oxygen supply. Mean CO exposures over a 24-hour period have been measured at 2-50 parts per million (ppm) among households that burn solid fuel, and often exceed the 9 ppm standard (in an 8-hour period) set by the WHO [34, 35]. CO levels in homes using solid fuels are sometimes high enough to result in blood carboxyhemoglobin (COHb) levels between 2.5% and 16%, with upper limits comparable to levels measured in heavy smokers [36, 37, 38].

Polycyclic aromatic hydrocarbons (PAHs) are a large class of organic compounds consisting of hydrogen and carbon organized in fused ring structures. PAHs are found on combustion-sourced particles as well as in the vapor phase of combustion emissions. Tobacco smoke is a major source of exposure to PAHs, but cooking and heating with solid fuels is also a major contributor to outdoor and indoor exposures in low resource countries [39-41].

High exposures to combustion by-products may impact fetal growth by several mechanisms, including interference with transplacental delivery of oxygen, which may cause fetal growth retardation, a form of LBW. Due to physical characteristics (e.g. large surface area) and chemical composition (e.g. PAHs adhering to surface of particle), small particles ($PM_{2.5}$) can induce oxidative stress, which causes local inflammation in the maternal pulmonary system, leading to both short-term and chronic damage to the lung [42]. Lung damage decreases maternal oxygen exchange, ultimately affecting oxygen transport to the fetus. In addition, the smallest particles can be transported from the lung across the alveolar-capillary membrane into the blood circulation, where they may exert effects on the cardiovascular system [43]. PAHs are transported by diffusion across membranes in the respiratory and gastrointestinal tract and are absorbed into the bloodstream. PAHs are capable of crossing the placenta [44, 45]. Umbilical cord blood PAH-DNA adducts have been associated with decreased fetal growth, after adjusting for maternal environmental tobacco smoke exposures in China [46], Krakow, and New York [47, 48]. Carbon monoxide is a potent fetotoxicant that binds with both maternal and fetal hemoglobin, forming carboxyhemoglobin and making oxygen less available for oxygenating tissue (such as the placenta). Exposures to low, constant CO *in utero*, as measured by maternal carboxyhemoglobin, produce large decrements in oxygen tension in the fetal blood stream [49].

In animal models, maternal exposure to ambient concentrations of elevated CO has been associated with poor fetal development including reduced birth weight [50-52].

Air pollutants are damaging xenobiotic substances in and of themselves, but they also induce harmful endogenous byproducts of oxidative stress [53, 54]. Genes that modulate oxidative stress are good candidates for investigating the interaction between air pollution and adverse human health. Glutathione S-transferases (GSTs) are a family of detoxifying enzymes that play an important role in protecting cells from reactive oxygen species (ROS), which can cause oxidative stress. GST enzymes conjugate ROS with glutathione, thus allowing the detoxification and excretion of harmful substances, such as ROS. A recent systematic review found suggestion of gene-environment interactions between outdoor air pollution and GST polymorphisms on respiratory lung function, but evidence for specific pollutants acting in concert with specific genes was not conclusively determined [55]. The complete deletion of both alleles (null polymorphism) confers absence of an important detoxifying enzyme, thus potentially increasing individual susceptibility to air pollutants.

Our aim was to evaluate whether the null polymorphisms of GSTM1 and GSTT1 genes in infants or mothers would modify the association between exposure to household air pollution (HAP) from wood fuel use and newborn birth weight. Our *a priori* hypothesis was that between the low and high HAP exposure groups, we would find differences in birth weight in mothers and/or infants with null genotypes compared to those with non-null

genotypes. This is a sub-study of a larger birth cohort reported elsewhere [10], which found that rural, Guatemalan mothers randomized to receive a well maintained chimney stove during pregnancy had a 39% reduction in personal exposures to carbon monoxide and had infants who weighed 89 grams more than infants whose mothers used open fires during pregnancy. Here we report on the cohort of pregnant women and children who participated in two studies that measured the impact of a randomized chimney stove on child pneumonia and pulmonary growth: the RESPIRE (Randomized Exposure Study of Pollution Indoors and Respiratory Effects) study [56] and the follow-up study CRECER (Chronic Respiratory Effects of Early Childhood Exposure to Respirable Particulate Matter) [57].

2. Materials and Methods

2.1. Study population

The study population, located in 23 rural communities in the Western highlands of Guatemala, consists of primarily indigenous Mam-speaking women and children. The majority of the households in these communities used wood fuel for cooking during the study period. Between October 2002 and May 2003, 266 pregnant women who used open fires were enrolled into the RESPIRE study; half were randomized to receive a vented chimney stove. Among women recruited during pregnancy, 254 singleton, healthy newborns were born (five miscarriages, four stillbirths, two pregnancies with multiple gestations, and one child with Down syndrome were excluded). We were able to measure birth weight on 224 (88%) newborns within 1 week and 190 (75%) within 48 hours of a home delivery. Between January 2007 and October 2008, a subset of these mothers and their children continued to participate in the follow-up study, CRECER. They provided saliva samples for DNA extraction. Our final sample includes those who had birth weight measured at less than 48 hours during the RESPIRE study (n=190) and 132 mother-infant pairs who provided infant genotypes (n=130) and/or maternal genotypes (n=116) during the CRECER follow-up study. Demographic characteristics and exposure data for women and infants in the present analysis were not statistically different from the 254 pregnant women who originally enrolled in the RESPIRE stove intervention trial or the 190 women who had infants weighed in their home within 48 hours of birth [10].

The study received approval from the institutional review boards at the University of California, Berkeley and the Universidad del Valle in Guatemala and was conducted in accordance with international guidelines for the protection of human subjects. Before collection of saliva DNA, all adult subjects provided written consent for the use of DNA for research purposes. The mother of the child provided consent for herself and for her child. All children were between 5 and 7 years of age at the time of salivary DNA collection. Local Mam-speaking trained fieldworkers explained the study in simple terms and all questions were answered before consent was signed.

2.2. Birth weight

Recruitment and follow-up of pregnant women and birth weight measurement of their newborns has been described previously [10]. Birth weight was measured in grams using a

calibrated Siltec BS1 baby scale with 10-gram readability (model 0309, Dogain Instruments, Inc; Santa Clara CA, USA).

2.3. Exposure to household air pollution

Pregnant women were eligible to participate in the RESPIRE study if they used an open fire for cooking. The study intervention, a chimney stove referred to as the *plancha*, was constructed in homes that were randomized to receive the intervention. All participating households were visited weekly by trained fieldworkers for health surveillance, during which time the stove was inspected, cleaned and, if necessary, repaired [58]. Personal exposures to CO were used as a surrogate measure for PM exposures [59, 60]. As described previously [58], we used high-range passive-diffusion colorimetric CO tubes (range, 1.04–2,000 ppm-hr) prior to randomized stove construction and low-range tubes (range, 0.4–400 ppm-hr) for measurements after construction (Gastec Corp., Kanagawa, Japan). Pregnant women wore CO tubes for a 48-hr period, with a total of 181 CO tubes worn before stove construction when all houses were using open fires. During this period, CO tubes were worn by 14% of women in the first, 8% in the second, and 78% in the third trimester. Because the purpose of the RESPIRE study was to measure CO exposures in infants, 22 pregnant women wore CO tubes after baseline but before the infants were born, with 14% of women in the first, 14% in the second, and 72% in the third trimesters. During the initial measurements when all houses used open fires, personal 48-hour CO measurements were 2.86 ppm (SD, 2.57). After stove installation, pregnant women in open fire households had average CO measurements of 3.28 ppm (SD 1.90) and women in chimney stove homes had average CO measurements of 1.94 ppm (SD 1.94), which represents a 60% relative reduction in exposures to HAP. Because we had inconsistent measurement of CO across all pregnant women in the study, but the stove was randomized and weekly visits to households were made to ascertain stove use and to examine and repair the stove, we use the stove as an indicator of exposure to HAP.

2.4. DNA collection and genotyping

DNA for genotyping was collected using saliva, as blood sampling was not culturally acceptable to this study population. Both mother and child were asked to rinse their mouth to remove food particles and then wait several minutes prior to saliva collection. Subjects were given a small amount of sugar to increase saliva production prior to collection. Subjects expectorated the saliva into the Oragene© (DNA Genotek Inc., Ottawa, Ontario, Canada) container until the amount of expectorant reached the marked level inside the unit (~2 mL). The Oragene collection unit contains a stabilizing solution; the sample does not require freezer/cooler storage. The kits were transported to the Holland Lab of University of California, Berkeley for processing. DNA of all samples was isolated following the Oragen protocol. Picogreen Quantification was done to determine the DNA concentration of the isolated DNA samples using a Microplate Fluorescence Reader (FLx 800, Bio-Tek Instruments, Inc.) and KC Junior software program (Biotex Instruments). DNAs were normalized to a concentration of 10ug/ml, plated and stored at –20°C.

The DNA isolation success rate from saliva samples in this study population was 97.6% for mothers and 95.3% for children. The mean DNA concentration was 74ng/μl ±67 for mothers

and 25ng/ μ l $\pm$ 30 for children. The mean total DNA yield was 22.35 μ g per subject (range from 0.5 μ g to 216 μ g). Children had lower yields and slightly lower success rate of isolation for two reasons 1) less cooperative with the sampling technique, and 2) children tend to exfoliate less than adults. Samples with sufficient good quality DNA were genotyped for GSTM1 and GSTT1 deletion polymorphisms using the Qiagen Multiplex Polymerase Chain Reaction (PCR) kit with some modifications [[61]. DNA primer pairs for GSTT1 and GSTM1 amplification are as follows: GSTT1 F: 5'-CTTACTGGTCCTCACATCTC-3', GSTT1 R: 5'-CAGGGCATCAGCTTCTGCTTT-3', GSTM1 F: 5'-CTTACTGGTCCTCACATCTC-3', GSTM1 R: 5'-CAGGGCATCAGCTTCTGCTTT-3'. As an internal positive control to verify DNA amplification in double null subjects, a 212 bp section of the albumin gene was co-amplified using the primers: ALB F: 5'-GACCAGCACCGACCACTATT-3', ALB R: 5'-AGAACAGGACAATGGGCAAC-3'. For each reaction, 100ng DNA (10ul of 10ug/ml) was amplified in a 50ul volume containing: 5X Q solution, 10x Coral Load PCR Buffer, 2mM MgCl₂, 0.1uM each of the 1x primer (5pmol), 2.5 unit (0.25ul) of Tag Polymerase, and 1ul of dNTP(10mM). In addition, 1unit (0.5ul) of Uracil-N-Glycosylase (Applied Biosystem) was added to prevent carry-over contamination. Gene fragments were simultaneously amplified using 96-Well GeneAmp® PCR System 9700 from Applied BioSystems (Carlsbad, California, USA), using the following cycling conditions: 15 min at 95C, [30 sec at 94C, 90 sec at 57-63C, 90 sec at 72C] $\times$ 32 cycles, 10 min at 72C. The null GSTM1 and GSTT1 genotypes were detected by the absence of a band at 267 bp and 434 bp, respectively, after electrophoresis and visualization on a 3.5% agarose gel stained with ethidium bromide.

For quality assurance, two positive and two negative controls were used. Laboratory standard DNA with four genotype combinations for the presence and absence of GSTM1 and GSTT1 bands were run with each experiment. The albumin gene was used as another internal positive control for the success of the amplification reaction. Sterilized deionized water was incorporated as the negative control. The quality control procedures also included duplicates and repeats of 5% of randomly selected samples on a separate gel. A minimum of three DNA ladders was used to improve precision reading of PCR products.

2.5. Statistical methods

Our dependent variable of interest was newborn birth weight, measured continuously in grams. Our primary independent variables of interest were the interaction of the stove type used during pregnancy (either the chimney stove intervention or open fire) and the maternal and infant GSTM and GSTT polymorphisms. Stove type is used as a surrogate for HAP exposure. We examined the independent and combined effects of stove type and genetic polymorphisms on newborn birth weight. We first constructed separate simple linear regression models with birth weight as a continuous outcome and stove type (improved chimney stove or open fire use during pregnancy) and the four gene polymorphisms (maternal or infant GSTT and maternal or infant GSTM) as dichotomous independent variables (present, null) to test for independent effects of stove and each of the four possible genotypes. Two separate models looked at the presence of both maternal and infant GSTT (both present, both null) and maternal and infant GSTM (both present, both null). The final model tested a composite risk score variable comprised of four categories which combined

the genetic polymorphisms (ranging from 1-4, where 1 represented presence of one copy of the GST alleles and 4 represented four present copies of either infant or maternal GSTT or GSTM; 0 was omitted because none of the mother-child pairs had 4 double-null alleles). For the multivariate regression models, we examined other potentially influential covariates using Student's t test and ANOVA. Because other factors influence birth weight, covariates were included in a multivariable linear regression model if they were statistically related to the exposure (stove type) or the outcome (birth weight, continuous scale) at $p < 0.20$. In order to avoid multiple comparisons and subsequent increase in Type II error, all models were adjusted for maternal systolic blood pressure at baseline, maternal height, weeks pregnant at time of recruitment, exposure to secondhand smoke (SHS), stove in sleeping area, and season at time of birth (cold, dry season, which represents harvest time vs. warm, dry and warm, wet seasons, which are times of food scarcity). Finally in all of the models, we tested whether the association between stove type and birth weight was modified by the maternal and infant polymorphisms, an interaction term (gene*stove) was introduced into all of the gene polymorphism models. Stata version 12 (StataCorp, College Station, Texas) was used for all data analyses.

3. Results

3.1. Study population

The study participants were 132 singleton infants who were weighed at birth during the RESPIRE study and for whom we have either maternal and/or child genotyping information performed during CRECER (**Table 1**). One quarter of the infants were low birth weight. Forty-eight percent of the pregnancies were closely spaced, with a prior delivery less than 24 months before the delivery of the study child. Seventy-seven percent of the pregnant women were of normal weight, with a body mass index (kg/m^2) during pregnancy between 18.5 and 25. One pregnant woman reported that she was a former smoker. Among the women who reported a smoker in the home, all smokers were male and smoked 1-2 cigarettes in a day.

Mothers and children in this ethnically homogenous indigenous population of Mayan descent have similar genotype frequencies for both GSTM1 and GSTT1. The frequency of the GSTM1 double-null allele was similar in 116 mothers and in 130 children (0.310 and 0.315, respectively). The GSTT1 double-null allele was less common overall but was similar in mothers and children (0.155 and 0.146, respectively). The distribution of allele frequencies for both genes fit the Hardy-Weinberg equilibrium ($p > 0.1$ by Pearson χ^2 test).

3.2. Birth weight by stove type and genetic polymorphisms

Low birth weight ($< 2,500$ grams) was 22.7% in this birth cohort. There was no difference in the weight of newborns born to mothers who used a chimney stove compared to newborns born in open fire households (**Table 1**). Among the 130 infants and 116 mothers who were genotyped for four possible combinations of present/null GSTT1 and GSTM1 polymorphisms, only the null polymorphism in maternal GSTM1 was statistically associated with a 144 gram reduction in birth weight (95% CI: -291, 2) in an unadjusted model (**Table 2**). None of the other maternal or infant null polymorphisms was statistically significant in either the unadjusted or adjusted models. With the composite scores that examined the

strength of association of combined maternal and infant GSTT1 or combined maternal and infant GSTM1 with birth weight, only the combined maternal/infant null GSTT1 achieved statistical significance in an adjusted model (-156 grams: 95% CI -673, -22) after controlling for systolic blood pressure, maternal height, weeks pregnant at recruitment, SHS exposure, stove in sleeping area, and season at time of birth. The fifth adjusted model, which examined a composite score, showed significant decreases in newborn birth weight if one (-347 grams: 95% CI -673, -22) of the maternal and infant GST genes were not expressed as compared to those with all four genes expressed.

In **Figure 1**, the effect modification of the maternal and infant genotypes in the presence of a chimney stove is displayed. None of the associations showed a relationship between the genetic polymorphism and the protective effect of the chimney stove. In the composite score, there was a trend toward higher birth weights with increasing number of expressed maternal or infant GST genes in the open fire group, although these findings were not statistically significant.

4. Discussion

To our knowledge, this is the first study to investigate the association between GST polymorphisms and household air pollution on newborn birth weight among a population highly exposed to wood smoke from cooking fires. This is also one of the first studies to examine the independent and combined effects of both infant and maternal genetic polymorphisms on newborn birth weight. We found that the complete deletion of both alleles (null polymorphism) in maternal GSTM1, the deletion both alleles in combined maternal and infant GSTT1, and the expression of only one of the four possible GST genes (as compared to the expression of all four GST genes), were associated with decreases in infant birth weight. When examining the impact of chimney stove in combination with genetic polymorphisms that may have a protective effect, we did not see a stronger relationship. However, among open fire users we saw an increasing birth weight trend with increasing number of GST alleles expressed in infants and mothers, which may signal an independent protective effect of the gene with higher exposure levels.

Although 26% of the women in the study were exposed to secondhand smoke, 92% of them reported that their spouse smoke cigarettes rarely. Our findings differ from a study that looked at the effects of GST polymorphisms and SHS on birth weight [15]. The authors found that among 266 Korean women, maternal GSTM1 and GSTT1 null genotypes did not have an independent effect on newborn birth weight, but birth weight was reduced by 245 grams (95% CI, -3 to -494 grams) among mothers with GSTM1-null genotype who were exposed to SHS.

We found an independent gene effect in our adjusted model that examined lack of expression of GSTT1 in both mother and infant, as well as when our composite score indicated that only one out of four possible GSTT1 and GSTM1 genes were expressed among infant/maternal pairs. There was no modifying effect on birth weight among mothers exposed to household air pollution from cooking fires. In contrast to our study, Wang et al. found a modifying effect of the maternal GSTT1-null genotype on the relationship between

active tobacco smoking during pregnancy and low birth weight among a multi-ethnic sample of 741 maternal-infant pairs in urban United States [17]. Continuous maternal smoking during pregnancy was associated with a mean reduction of 377 grams in birth weight compared to birth weight of infants to mothers who never smoked ($p<0.001$). When GSTT1-null genotype was added, the estimated reduction in birth weight was 642 grams ($p<0.001$).

The strengths of the current study are several-fold. A population known to have high exposures to household air pollution from cooking fires provided a unique opportunity to study the effects of maternal and infant genetic polymorphisms related to oxidative stress. A handful of studies have looked at secondhand and active tobacco smoke exposures, but this is the first study that we know of to assess potential effect modification of maternal and infant genotypes on exposure to wood smoke and newborn birth weight. Second, the exposure (stove type) was randomized at the beginning of the RESPIRE study, and the two groups were otherwise similar at baseline, as has been previously demonstrated [62]. We conducted weekly home visits to monitor stove maintenance and use, which provides us with evidence that the stove was consistently used and representative of exposure to HAP in two distinct groups. Finally, our research sample was drawn from an ethnically homogeneous, indigenous population. We have minimized potential confounding bias present in other studies where different race and ethnicities have different distributions of genetic polymorphisms and also have different adverse birth outcome risks.

The GSTM1 and GSTT1 null allele frequencies for this Guatemala cohort were 31% and 15%, respectively and are similar to other admixed *mestizo* populations (descendants of European and Native American races) in Latin America. Latin American populations have lower GSTM1 null frequencies (ranging from 38-49%) and lower GSTT1 null frequencies (9%-15%) [63-66] than Caucasians (GSTM1-null ranging from 51-55% and GSTT1-null between 38 and 58%) [55, 67] and Asians (GSTM1-null ranging from 40-58% and GSTT1-null of 47%) [55]. Populations of African descent in the Americas have lower GSTM1 null frequencies (16-35%) than GSTT1 null frequencies (22-44%) [67-69]. Since this Guatemala cohort is an indigenous population of Mayan ancestry, the similarity in frequencies to *mestizo* Latin American populations was expected. Data on ethnic distribution of GSTM1 and GSTT1 null genotypes among populations in the world may be useful in targeting populations at risk for adverse health effects from exposures to environmental toxicants.

The small sample size restricted our ability to find a modifying effect between individual metabolizing genes on the association between HAP and infant birth weight, but nevertheless we were able to detect reductions in birth weight in infants where both infant and the mother has the GSTT1 null genotype after adjusting for important covariates. Although most of the differences did not reach statistical significance, they were in the hypothesized direction. Because of the limited statistical power of our study, our findings need confirmation in a larger population.

A second limitation is the lack of consistently measured pollutant concentrations during the pregnancy period, especially during first and second trimester. Measured pollutants may have provided more information about which specific pollutants contribute to the LBW

outcome. Slama et al. found no evidence of effect modification between GSTM1-null and GSTT1-null polymorphisms and exposure to low concentrations of ambient particulate matter (aerodynamic diameter less than 2.5 μm) on newborn birth weight in 386 German infants [22]. The glutathione S-transferase (GST) enzymes metabolize and detoxify PAHs, but we do not have individual levels of air pollutants and instead use stove type to represent a mixture of air pollutants. An exposure-response function that quantified the duration and intensity of exposures to PM and PAHs would have provided more precision for our estimates of exposure to HAP.

A third limitation is our lack of information about dietary intake among pregnant women in the study. On an aggregate level, women in the study were from households primarily engaged in subsistence farming, with the primary crop being corn. Focus groups conducted with pregnant women in the rural study region in 2005 found that women believe that taking vitamins and increasing food intake during pregnancy will lead to complicated deliveries due to “large babies”. Women in these focus groups described a diet that was very homogeneous, consisting of corn at each meal, in the form of corn porridge, tamales, or tortillas, and supplemented with a small amount of stewed green vegetables and a protein, usually an egg or a small piece of chicken [70].

A final limitation is candidate gene selection which was limited to GSTM1 and GSTT1. Other genetic polymorphisms also may be responsible for modifying the effect between the exposure and the outcome. However, the GST null polymorphisms have been extensively studied in relation to air pollution and birth weight and were thus well-suited for the present study.

5. Conclusion

Screening for genetic polymorphisms that may modify the relationship between household air pollution and birth weight is obviously not feasible in most population studies around the world. However, the public health implications of a differential risk of susceptibility are significant. GST polymorphisms are common within the described population of Guatemalan mothers and infants and this is true for other populations. Although we were not able to demonstrate statistically significant modification of the effect of exposure to wood smoke, we did find a GST gene direct effect associated with lower infant birth weight. Women are highly exposed to HAP from cooking fires, and most of the low birth weight effect occurs among children born to women in low resource countries who are exposed to these fires. In the 2010 Global Burden of Disease comparative risk assessment, HAP was the 2nd highest risk factor for ill health among women of all ages, including women during their child-bearing years. Among neonates, HAP was estimated to cause 100,000 deaths attributed to acute lower respiratory infections deaths in 2010 [71]. These estimates would certainly be much higher if they included neonatal deaths due to prematurity and fetal growth retardation, two important contributors of low birth weight. Global efforts should prioritize the implementation of clean cook stove programs and provide education on reducing personal exposure to harmful air pollution.

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- This is the first study to evaluate whether null polymorphisms of GSTM1 and GSTT1 genes in infants or mothers modify the association between exposure to household air pollution (HAP) from wood fuel use and newborn birth weight.
- Maternal null GSTM1 was associated with a 144 gram (95% CI: -291, 1) decrease in birth weight
- Combined maternal/infant null GSTT1 was associated with a 155 gram (95% CI -303, -8) decrease in birth weight
- Effect modification by chimney stove use was not demonstrated

Figure 1.
Adjusted interaction effects of differences in birth weight based on GST genetic polymorphisms among chimney stove users

Table 1

Characteristics of mothers and infants among pregnant women who delivered singleton infants

Characteristic	Stove type		
	Chimney stove	Open fire	<i>p</i> -value
Genetic polymorphisms			
Maternal GTSM1, number (%) (n=116)			0.22
Null	11 (24.4)	25 (35.2)	
Present	34 (75.6)	46 (64.8)	
Infant GTSM1, number (%) (n=130)			0.71
Null	18 (33.3)	23 (30.3)	
Present	36 (66.7)	53 (69.7)	
Maternal GSTT1, number (%) (n=116)			0.28
Null	9 (20)	9 (12.8)	
Present	36 (80)	62 (87.3)	
Infant GSTT1, number (%) (n=130)			0.34
Null	6 (11.1)	13 (17.1)	
Present	48 (88.9)	63 (82.9)	
Birth outcomes			
Birth weight (n=132)			0.75
< 2,500 grams, number (%)	13 (24.1)	17 (21.7)	
≥ 2,500 grams, number (%)	41 (75.9)	61 (78.2)	
Grams, mean ± SD	2751 ± 366	2757 ± 386	0.92
Sex of child, number (%)			0.62
Female	26 (48.2)	41 (52.6)	
Male	28 (51.8)	37 (47.4)	
Season of delivery			0.09
Dry, cold	7 (13)	13 (16.7)	
Dry, warm	9 (16.6)	4 (5.1)	
Rainy, warm	38 (70.3)	61 (78.2)	
Household Characteristics			
Paternal age, years, mean ± SD	28.8 ± 7.6	30 ± 7.5	0.34
Paternal education, years, mean ± SD	4.7 ± 3.2	4.3 ± 3.2	0.52
House altitude, meters, mean ± SD	2597 ± 183	2599 ± 192	0.93
Stove in same room as bedroom, number (%)	11 (20.4)	9 (11.5)	0.16
Uses fire for heating home, number (%)			0.23
Yes	42 (77.8)	67 (85.9)	
No	12 (22.2)	11 (14.1)	
Tobacco smoking in home, number (%)	10 (18.5)	24 (30.7)	0.11
Traditional steam bath (temascal), number (%)	49 (90.7)	74 (94.9)	0.35
Dirt floor in main house, number (%)	51 (94.4)	70 (89.7)	0.32
Has electricity in main house, number (%)	39 (72.2)	54 (69.2)	0.71
Economic support ^a , mean ± SD	4.8 ± 2.4	4.8 ± 2.3	0.47

Characteristic	Stove type		
	Chimney stove	Open fire	<i>p</i> -value
Crowding ^b , mean ± SD	7.6 ± 2.8	7.4 ± 2.8	0.62
Total assets ^c , mean ± SD	1.4 ± 0.8	1.3 ± 0.9	0.38
Pregnancy characteristics			
Maternal age, years, mean ± SD	26.2 ± 6.9	27.6 ± 6.8	0.22
Maternal education, years, mean ± SD	2.7 ± 2.3	2.1 ± 2.3	0.13
Weeks pregnant at baseline exam ^e , mean ± SD	27.1 ± 7.7	30.3 ± 6.9	0.02
First pregnancy, number (%)	3 (5.5)	7 (8.9)	0.45
Child spacing ^f , number (%)	23 (42.6)	40 (51.3)	0.32
Gravidity, mean ± SD	5.2 ± 2.8	5.5 ± 2.7	0.59
Live births, mean ± SD	3.5 ± 2.3	3.6 ± 2.2	0.95
Maternal height in cms, mean ± SD	144.1 ± 4.8	143.3 ± 4.0	0.16
Body Mass Index during pregnancy in kg/m ² , mean ± SD	23.7 ± 2.1	23.5 ± 2.1	0.50
Blood pressure during pregnancy, systolic, in mmHg, mean ± SD	106.4 ± 7.8	108.5 ± 9.6	0.08
Blood pressure during pregnancy, diastolic, in mmHg mean ± SD	66.1 ± 7.0	68.5 ± 7.8	0.21

^d Animal index is a 0-4 item measure of reported ownership of cattle, horse/mules, sheep or pigs

^a Ratio of household dependents/ household workers

^b Ratio of people/room

^c Asset index is a 0-6 item measure of reported ownership of radio, television, refrigerator, bicycle, motorcycle or car/truck

^e Fundal height in cm measured by physician at first prenatal physical examination

^f Has older sibling 24 months old

Table 2

Estimated independent effect of stove type, maternal and infant genetic polymorphisms 18 on newborn birth weight measured within 48 hours of birth.

	Birth weight, Mean (SD), g		β (g), unadjusted [95% CI]		β (g), adjusted [95% CI] ^a	
	n	(%)	Grams	SD		
Stove type	132					
Open fire	78	(59.1)	2757	(386)	6 [-126, 138]	-7 [-142, 127]
Chimney stove	54	(40.9)	2751	(367)	ref	ref
Maternal GSTM1	116					
Null	36	(68.9)	2661	(406)	-144 [-291, 2]	-85 [-240, 70]
Present	80	(31.1)	2805	(351)	ref	ref
Infant GSTM1	130					
Null	89	(68.5)	2781	(473)	34 [-107, 176]	-10 [-154, 134]
Present	41	(31.5)	2747	(327)	ref	ref
Maternal GSTT1	116					
Null	18	(15.5)	2702	(341)	-68 [-258, 122]	-72 [-259, 114]
Present	98	(84.5)	2771	(379)	ref	Ref
Infant GSTT1	130					
Null	19	(14.6)	2705	(275)	-61 [-247, 125]	-134 [-328, 59]
Present	111	(85.4)	2767	(393)	ref	ref
Maternal and Infant GSTM1	117					
Both null	58	(49.6)	2744	(426)	-30 [-167, 106]	-12 [-127, 150]
Both present	59	(50.4)	2775	(311)	ref	ref
Maternal and Infant GSTT1	118					
Both null	36	(30.5)	2696	(305)	-97 [-243, 48]	-156 [-303, -8]
Both present	82	(69.5)	2793	(391)	ref	ref
Composite Score ^b	114					
One present	6	(5.3)	2475	(441)	-288 [-607, 32]	-347 [-673, -22]
Two present	22	(19.3)	2705	(420)	-58 [-251, 135]	-61 [-265, 143]
Three present	45	(39.5)	2833	(371)	70 [-88, 228]	23 [-136, 182]
Four present	41	(35.9)	2763	(326)	ref	ref

^a Adjusted for maternal systolic blood pressure at baseline, maternal height, weeks pregnant at time of recruitment, exposure to second hand smoke, stove in sleeping area, and season at time of birth

^b Based on combination of maternal and genetic polymorphism, score 1-4