Environmental Research 111 (2011) 1215–1221

Contents lists available at SciVerse ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Maternal smoking during pregnancy, polymorphic CYP1A1 and GSTM1, and lung-function measures in urban family children ☆

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ARTICLE INFO

Article history:
Received 24 August 2010
Received in revised form
29 July 2011
Accepted 2 August 2011
Available online 27 August 2011

Keywords:
Gene–tobacco interaction
Lung function
Maternal smoking
Metabolic genes

ABSTRACT

Purpose: Understanding the interplay between genes and in-utero tobacco exposure in affecting child lung development is of great significance. In this study, we tested the hypothesis that tobacco-related lung-function reduction in children differs by maternal polymorphic genes *Cytochrome P450 1A1* (*CYP1A1*) and *Glutathione S-transferase Mu 1* (*GSTM1*).

Materials and methods: Data were collected among 370 children (6–10 years old, 81.6% African-Americans) and their biological mothers visiting a large children's hospital. Study hypotheses were tested using multiple regression method.

Results: Among the study sample, 143 mothers smoked throughout pregnancy and 72 smoked on a daily basis. Spirometric measures (mean ± SD) included were: forced vital capacity (FVC) = 1635 ± 431 mL, forced expiratory volume in the first 1 s (FEV_1) = 1440 ± 360 mL, percent FEV_1/FVC ratio = 89 ± 12, and forced expiratory flow between the 25% and 75% of FVC (FEF_{25-75}) = 1745 ± 603 mL. In addition to a tobacco effect on FVC (−131 mL, 95% CI: −245, −17) and FEV_1/FVC ratio (42, 95% CI: 1, 83), regression analysis controlling for covariates indicated that for the subsample of children whose mothers were *CYP1A1*2A* homozygous, maternal daily smoking was associated with −734 mL (95% CI: −1206, −262) reductions in FEV_1 and −825 mL (95% CI: −909, −795) reductions in FVC; reduced smoking was still associated with −590 mL (95% CI: −629, −551) reductions in FVC. For children of mothers with *GSTM1* deletion, persistent daily smoking was associated with −176 mL (95% CI: −305, −47) reductions in FVC.

Discussion and conclusions: Maternal smoking during pregnancy was significantly associated with lung-function reduction in children, particularly for those whose mothers possessed the polymorphic *CYP1A1*2A* and GSTM1 deletion.

1. Introduction

An estimate of 400,000–800,000 (12–23%) pregnant women smoke in the United States (Ebrahim et al., 2000; Florek et al., 2004; Pickett et al., 2005), exposing themselves and their developing fetuses to tobacco smoke. Maternal smoking during pregnancy has been associated with multiple respiratory illnesses, including asthma (Alati et al., 2006; Infante-Rivard et al., 1999; Li et al., 2005), sudden infant death syndrome (Daltveit et al., 2003; Storm et al., 1999), infections (DiFranza et al., 2004; Mannino et al., 2001), and many other morbidities/mortalities (Adair-Bischoff and Sauve, 1998; Jurado et al., 2005; Malloy et al., 1988). Lung development emerges very early in life as major airway branching commences at around 5 weeks of gestational age (Hislop, 1995). Published studies reported that prenatal exposure to tobacco is associated with reductions in forced vital capacity (FVC), forced expiratory volume in the first 1 s (FEV_1), FEV_1/FVC ratio, and forced expiratory flow between the 25% and 75% of FVC (FEF_{25-75}) (Cunningham et al., 1994, 1995; Gilliland et al. 2000; Gilliland et al., 2003; Li et al., 2000; Palmer et al., 2006; Wang and Pinkerton, 2008). However, data are lacking on specific effects among inner-city children, an underserved urban population who may be particularly vulnerable to tobacco-related impact.

Although tobacco exposure may affect fetal and child health, not all exposed children suffer from the same tobacco-related adverse effects. Recent studies indicate that polymorphisms in a

Abbreviations: FVC, forced vital capacity; FEV_1, forced expiratory volume; FEF_{25-75}, forced expiratory flow between the 25% and 75% of FVC; SD, standard deviation; PCR–RFLP, polymerase chain reaction–restriction fragment length polymorphism; *CYP1A1*, *Cytochrome P450 1A1*; *GSTM1*, *Glutathione S-transferase Mu 1*

☆*Funding source*: This research was supported in part with funds from Children's Research Center of Michigan.

IRB approval: The protocol of the reported study was approved by the Human Investigation Committee at Wayne State University, Detroit, Michigan.

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doi:10.1016/j.envres.2011.08.003

number of xenobiotic metabolic genes may modify the negative consequences of maternal smoking on children (Breton et al., 2011; Gilliland et al., 2002; Infante-Rivard et al., 2000; Infante-Rivard et al., 2006; Jurado et al., 2005; Whyatt et al., 2000). *Cytochrome P450 1A1* (*CYP1A1*) is a common xenobiotic gene that encodes the phase-1 rate-limiting enzyme responsible for catalyzing metabolisms of a variety of chemicals including toxic tobacco chemicals (Georgiadis et al., 2005; Iba et al., 1998; Rand et al., 2006; von Schmiedeberg et al., 1999; Whyatt et al., 2000).

Among the reported variations in *CYP1A1*, the single base substitution polymorphism (*T6235C*) in the 3′ non-coding region of *CYP1A1* (**2A*) is prevalent, particularly among African descendants (Bartsch et al., 2000; Hung et al., 2003; Infante-Rivard et al., 2000). The *Msp*I recognizable mutation *T6235C* in *CYP1A1* results in three unique genotypes (*TT*, *TC*, and *CC*) and the genotypes *CC* or *TC* have been reported to exhibit increased enzyme activity relative to *TT* (Kiyohara et al., 1996; Landi et al., 1994). Findings from molecular epidemiological studies indicate that the *C* allele, in combination with maternal smoking during pregnancy, is associated with increased risk of premature birth, reduced birth weight/size (Delpisheh et al., 2009; Infante-Rivard et al., 2006; Sasaki et al., 2006; Wang et al., 2002), risk of sudden infant death (Rand et al., 2006), and orofacial clefts (Hozyasz et al., 2005; Lammer et al., 2005). Therefore, we hypothesized that the effect of maternal smoking on child lung function would be greater for children whose mothers carry a homozygous genotype with *C allele* relative to *TT*/*CC* genotypes.

Glutathione S-transferase Mu 1 (*GSTM1*) is another xenobiotic gene that encodes a phase-2 enzyme involved in the detoxification of hydrophobic electrophiles derived from phase-1 metabolism by catalyzing their conjugation to glutathione for excretion (Burim et al., 2004). A full deletion of the gene (*GSTM1 null*), resulting in a complete lack of enzyme activity, has been reported (Burim et al., 2004; Gilliland et al., 2002; Hung et al., 2003). Consequently, greater reductions in lung-function measures would be possible for children whose mothers are *GSTM1 null* after exposure to smoking during pregnancy.

In this study, we examined the association between maternal smoking during pregnancy, polymorphisms in the mothers' two metabolic genes *CYP1A1* and *GSTM1* and lung function in children. These two genes were selected because of the confirmed knowledge of their role in metabolizing xenobiotics, including tobacco toxicants, the established relation between polymorphic changes in the genes and enzyme activities, and high prevalence rates of the polymorphic changes in the study population, which are prerequisite for a study to examine interactions between a gene and tobacco exposure (Schulte and Perera, 1993).

2. Materials and methods

2.1. Participants and data collection

Eligible participants included 400 mothers and their biological children 6–10 years old who were seen by doctors at a very large Midwestern children's medical center. The hospital serves the greater Detroit area with children that are predominantly African-Americans and 80% of them are either Medicaid recipients or Medicaid eligible. Participants were recruited by a research associate in the outpatient clinic setting. One child per mother was invited to participate. For a few mothers with more than one eligible child, only one was selected by the mother after discussion between the mother and her children. Children with diagnosed respiratory infections, cardiovascular diseases, and any other conditions that might affect lung-function test were excluded. The study protocol was approved by the Human Investigation Committee at Wayne State University.

To better represent children visiting the hospital, data collection was conducted on a monthly basis from October 2006 to March 2008 to cover various periods of a year. One research associate was responsible for data collection to minimize data-collector bias. Approximately 80% of the approached mothers/children agreed to participate by signing the informed consent. Among the 400 eligible mother–child pairs who participated in the study, data for 370 pairs were included. Others were excluded because of inadequate spirometry data ($n=17$), lack of genotyping data ($n=12$), and incomplete survey data ($n=1$).

2.2. Maternal smoking

For mothers who responded positively to the question, "Have you ever smoked on a daily basis for at least 30 days in your life?", their smoking behavior during pregnancy was systematically assessed using the Life-History Calendar method (Freedman et al., 1988). For each of the following periods: (a) 30 days prior to pregnancy and (b) each of the three trimesters, mothers were asked "How frequently did you smoke during [each of the four periods]?" with answer options of "daily", "weekly", and "monthly". This was followed by the question "On the average, how many cigarettes did you smoke on a day when you smoked during [each of the four periods]?" Studies using biomarkers (Caraballo et al., 1998, 2004; Matt et al., 1999; Peterson et al., 1997), medical records (Rice et al., 2007), and prospective data (Pickett et al., 2009; Tomeo et al., 1999) have shown that self-reported smoking status (smoked or not) is more reliable than self-reported number of cigarettes smoked, including maternal smoking during pregnancy reported by mothers of school-aged children (Pickett et al., 2009; Rice et al., 2007; Tomeo et al., 1999). Our analysis indicated a highly skewed distribution with a large range (0–9000 cigarettes) of the amount of cigarettes smoked (number of cigarettes per day times the days smoked) during the period of pregnancy and the month prior to pregnancy, and 60.1% smoked zero cigarettes. To more effectively assess the relationship of maternal smoking and its interaction with genes in impacting child lung function, we elected to categorize mothers into three mutually exclusive groups—"*persistent daily smokers*": mothers who reported daily smoking throughout pregnancy; "*reduced smokers*": mothers who reported reductions in frequency and/or number of cigarettes smoked; and "*nonsmokers*": mothers who reported no smoking throughout the pregnancy.

2.3. Child urine cotinine

Urine cotinine levels of children were assessed using the NicAlert™ testing kit (Nymox Pharmaceutical Corporation, New Jersey). This semiquantitative method provides an easy-to-use, sensitive, and reliable tool (Bernert et al., 2005; Nymox Corporation, 2005). According to the standard from the manufacture, a test reading of 0 (equivalent to cotinine concentration of 0–10 ng/mL) indicates no exposure to environmental smoke, a reading of 1 (10–30 ng/mL) indicates light exposure, and a reading of 2 or higher ($\geq$30 ng/mL) indicates heavy exposure (Nymox Corporation, 2005). Although the half life of cotinine in children is approximately 72 h (Collier et al., 1994), our analysis indicated that urine cotinine was significantly associated with reported exposure to environmental tobacco smoke during the past month ($r=0.80$), the past year ($r=0.51$), between grade 1 and 1 year ago ($r=0.41$), and from birth to grade 1 ($r=0.37$). Therefore, we classified the children with urine cotinine test readings ≥ 1 as having exposure to environmental tobacco smoke. This objectively assessed variable was used as a proxy of postnatal exposure to environmental smoke in multivariate analysis.

2.4. Child pulmonary function

Pulmonary function tests were conducted by measuring spirometry in the Pediatric Pulmonary Laboratory at the hospital using the computerized 1085 Elite System (Medical Graphics Corporation, St. Paul, MN), and the software MedGraphics Breeze Suite (Medical Graphics Corporation, St. Paul, MN). Two senior and certified respiratory therapists conducted the testing according to the published guidelines of the American Thoracic Society and Artes et al. (American Thoracic Society, 1995; Arets et al., 2001; Eber and Zach, 2005). With no knowledge of the status of maternal smoking of individual participants, the leading author and the pulmonary specialist author examined the quality of the data from lung-function testing case by case against the computerized records. Cases with disqualified testing results were excluded from the analysis. Four spirometry measures were examined in this analysis: FVC, FEV_1, FEV_1/FVC ratio (%), and FEF_{25-75}.

2.5. Genotyping

Genotyping analysis of *CYP1A1* and *GSTM1* was conducted in the Molecular Epidemiology Research Laboratory at Henry Ford Health System, Detroit. Cell samples were collected following the 3-brush protocol (Abbas et al., 2004; King et al., 2002) using the buccal swab package (Epicenter Bio Tech, Valencia, CA). DNA was extracted using the QIAGEN QIAamp DNA Mini Kit (QIAGEN Inc, Valencia, CA). For samples with a limited yield of DNA, whole genome amplification was performed using the QIAGEN REPLI-g Kit (QIAGEN Inc.).

The single base substitution polymorphism (thymine to cytosine) in the 3′ non-coding region of the *CYP1A1* gene was detected using the polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) method (Sato et al., 1999) with minor modifications. DNA (80 ng) was amplified in a reaction volume of 30 μL