Effect modification by GST and TNF- α polymorphisms in the association between air pollution and lung function in asthmatic children

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Abstract

Background: Air pollutants induce the increase of inflammatory components in the lungs and the decrease in pulmonary function. Genetic polymorphisms may modify the inflammatory and immunological response to the inhalation of those pollutants. Objective: To investigate effect modification by GST and TNF- α polymorphisms in the association between particulate matter (PM₁₀) and lung function. Methods: This is a cross-sectional epidemiological study with 112 asthmatic children aged 6 to 14 years old. GSTM1, GSTT1, GSTP1, and TNF- α polymorphisms were genotyped using the polymerase chain reaction (PCR) technique. Spirometry tests were conducted. We tested associations using generalized linear models, adjusting for confounding variables. The effect modification of the genetic polymorphisms was assessed, including the genotypes and pollutants. Results: In carriers of the TNF- α -308 GA or AA genotypes, we found a reduction of 11.5% (95% CI: -19.08, -3.30) in FEV₁ in the current exposure to PM₁₀ and a reduction of 10.46% (95% CI: -14.91, -5.77) for the 5-day cumulative exposure. The TNF- α -1031 TT genotype was associated with a decline in FEV₁ and FEF_{25-75%} for the cumulative exposure to PM₁₀. The GSTP1 wild-type genotype was associated with a decrease in FEF_{25-75%} of 23.45% (95% CI: -30.6, -15.56) and in FEV₁ of 13% (95% CI: -15.21, -10.74). We found that among GSTT1 null children, the cumulative exposure to PM₁₀ was also associated with a reduction in FEV₁, FVC, and FEF_{25-75%}. Conclusion: Genetic polymorphism in GST and TNF- α may modify the association between particulate matter and pulmonary function in asthmatic children and adolescents.

INTRODUCTION

Asthma is a chronic inflammatory disease that affects approximately 339 million people worldwide, representing the main chronic disease among children¹. The pathogenesis of asthma can involve complex relationships between environmental, behavioral, and genetic factors². Among the environmental factors, consistent evidence indicates that exposure to gaseous and particulate pollutants may induce or aggravate asthma³. Although the mechanisms underlying the relationship between air pollution and asthma are not fully understood, potential physiological pathways may include induction of airway inflammation and oxidative stress, failures in bronchial epithelial barrier function, and alterations in the immune responses^{4,5,6}.

These mechanisms can contribute to bronchial hyperreactivity, pulmonary remodeling, and reduced lung function.

Previous studies have shown that the effects of air pollution on respiratory outcomes may vary according to age, gender, ethnicity, and socioeconomic status^{3,7}. Genetic and epigenetic mechanisms may also contribute to susceptibility to the respiratory effects of air pollution by association with the biological response to air pollutants, such as oxidative stress and inflammatory pathways.

Among the several candidate genes related to antioxidative stress systems, particular attention has been paid to the genes of glutathione S-transferases (GST)^{8,9}. GST genes encode for phase II xenobiotic-metabolizing enzymes that participate in cellular defense against chemically induced toxicity and oxidative stress¹⁰. It has been hypothesized that GST polymorphisms that alter GST function may lead to increased or decreased susceptibility to toxic environmental agents.

Polymorphisms of genes encoding tumor necrosis factor-alpha (*TNF* - α) have also been investigated as potential modifiers of the effects of air pollution on asthma outcomes¹¹. *TNF* - α is a pro-inflammatory cytokine that plays a critical role in host defense against infection. The single nucleotide polymorphism (SNP) *TN Φ -a* -308 *T*>*A* has been associated with higher production of *TNF* - α and the subsequent amplification of the inflammatory response to oxidizing agents, which may contribute to susceptibility to induction and exacerbation of asthma^{12,13}.

Although previous reviews have reported that GST and *TNF* - α polymorphisms may interplay with the effects of air pollution on respiratory outcomes, the results are highly heterogeneous across studies^{8,9,11,14}. In addition, few studies have investigated genetic susceptibility to the effects of particulate matter on lung function.

In this study, we investigate effect modification by the polymorphism of *GSTM1*, *GSTT1*, *GSTP1* and *TN Φ -a* genes in the association between particulate matter (PM₁₀) and pulmonary function in asthmatic children and adolescents.

MATERIALS AND METHODS

This cross-sectional study was carried out from November 2015 to December 2016. The initial sample included 133 children and adolescents aged between 6 and 14 years old, with a medical diagnosis of asthma, residing in the city of Rio de Janeiro. Participants were recruited from routine consultations at outpatient clinics that provide specialized asthma care at two municipal health centers. Children and adolescents with severe asthma and other pulmonary pathologies were excluded from the study. After the guardians signed the consent form, study participants underwent spirometry and anthropometric tests and had oral cells collected. The final study sample included 112 participants.

Pulmonary function

Spirometry was performed with the Koko® portable spirometer. The test was carried out according to the criteria established by the American Thoracic Society (1995)¹⁵. Spirometric parameters included: forced expiratory volume in the first second (FEV₁), forced vital capacity (FVC), and forced expiratory flow between 25% and 75% of vital capacity (FEF_{25-75%}). The results were expressed as a percentage of the predicted value.

Covariate data

Covariate data were collected through a structured questionnaire completed by the parent or guardian of each child. The questionnaire included information on sex, age, presence of asthma symptoms in the last four weeks, family income, maternal education, passive smoking, and use of corticosteroids.

Exposure assessment

Daily concentrations of particulate matter (PM₁₀) were obtained from the Municipal Environment Department of Rio de Janeiro (SMAC). Exposure was calculated as the average concentration of the monitoring sites located closest to the participants' residence. The mean daily concentrations of PM₁₀ were calculated for the same day of the spirometry test (thus representing current exposure or lag 0 exposure) and up to 5 days before the measurement of pulmonary function (5-day lagged exposures).

Genotyping

Genomic DNA was obtained from exfoliative cells by swabbing the oral mucosa. For DNA extraction, we used the Invisorb Spin Swab® kit (Invitrogen). The genotyping of *TNF* α -1031T>C (rs1799964), -308G>A (rs1800629) and *GSTP1*(Ile105Val - rs1695) polymorphisms was performed using the polymerase chain reaction (PCR) technique in real time with the aid of TaqMan probes (Applied Biosystems, Foster City, CA, USA). The genotypes of *GSTM1* and *GSTT1* were characterized using the multiplex PCR technique¹⁶. The absence of PCR products for *GSTM1* or *GSTT1* in the presence of bands of the internal control CYP1A1 was indicative of the null genotype. The subjects were classified as either present (+), when at least one specimen of the gene was detected, or null (-), when they showed absence of the gene.

Statistical analysis

Linear regression models and polynomial distributed lag models (PDL) were used to assess the association between exposure to particulate matter and the logarithm of pulmonary function. Effect estimates are presented as the percentage change in the parameters of pulmonary function for each increase of 10 $\mu\text{g}/\text{m}^3$ in PM₁₀ exposure on the day of the spirometry test and up to 5 days lag.

We used causal directed acyclic graphs (DAG) to select a sufficient adjustment set of variables that would allow the identification of the total and cumulative effect of PM₁₀ on pulmonary function. Potential confounders included common causes of both exposure and outcome and predictors of lung function. The sufficient adjustment set was identified using the DAGitty application¹⁷ and included the variables sex, age, socioeconomic status, height, and ambient temperature (at 5-day lag). We used the same adjustment set of covariates for all models, and maternal education was used as a proxy for family socioeconomic status (Figure 1).

The Hardy-Weinberg Equilibrium (HWE) was calculated for all polymorphisms and evaluated using the Chi-square method. Effect modification was assessed by stratified analyses according to genotype groups.

The GST polymorphisms were encoded as *GSTP1* Ile105 wild type (AA or Ile/Ile - dominant homozygote) or Val105 variant (AG or Ile/Val - heterozygote or GG or Val/Val - homozygote); *GSTM1* and *GSTT1* were genotyped as present +/+ (absence of polymorphism) or null -/- (presence of polymorphism). The *TNF* α -1031 T-C into TT (Wild type homozygous) *versus* TC (heterozygous variant) or CC (homozygous variant) and *TNF* α -308G-A into GG (Wild type homozygous) *versus* GA (heterozygous variant) or AA (homozygous variant). All the analyses were performed using the R software (The R Project for Statistical Computing) and the packages X, Y and Z.

RESULTS

A total of 133 children and teenagers with a medical diagnosis of asthma were recruited. Of these, 21 were excluded from the analysis because they did not undergo the spirometric test according to the ATS standardization (1995) or their home address was not georeferenced (Figure 2). Of the 112 children and adolescents included in this study, 67.9% had a monthly family income lower than or equal to two minimum

wages, and 32% were exposed to secondhand tobacco smoke. The average age of the population was 9 years old. Approximately 32% of the sample reported asthma symptoms in the last four weeks, and 83.9% were using regular inhaled corticosteroids for asthma treatment (Table 1).

All the studied polymorphisms were in Hardy-Weinberg Equilibrium. The distribution of *TNΦ-a*-308 and *TNΦ-a*-1031 homozygous variant genotypes was 0.9% and 11.6%, and the distribution of *GSTP1*, *GSTM1* and *GSTT1* antioxidant genes was 16.1%, 35.7% and 30.4%, respectively (Table 1).

During the study period, the annual average concentrations of PM₁₀ exceed the guideline value of 20 µg/m³, with an average of 38.6 µg/m³, and exceeded the reference limit of 50 µg/m³ for daily average in 16.3% of the days (65 days).

Associations between PM₁₀ and pulmonary function according to genotype

Among children and adolescents with *TNΦ-a*-308 GA or AA genotypes, we found a reduction of 11.5% (95% CI: -19.08, -3.30) in FEV₁ for each increase of 10 µg/m³ in the current exposure to PM₁₀ and a reduction of 10.46% (95% CI: -14.91, -5.77) for the 5-day cumulative exposure. Among those with the *TNΦ-a*-308 GG genotype, the reduction in FEV₁ was lower both for current (-1.84; 95% CI: -4.75, 1.17) and cumulative exposure (-5.47; 95% CI: -6.80, -4.12) (Figure 3; E-table 1).

Among participants with the *TNΦ-a*-1031 TT genotype, the cumulative exposure to PM₁₀ was associated with a higher decline in FEV₁ (-7.13; 95% CI: -9.25, -4.95) compared to those with *TNΦ-a*-1031 TC or CC genotypes (-4.59; 95% CI: -6.50, -2.64). In these subgroups, the same pattern was observed for the FEF_{25-75%} parameter (Figure 3; E-table 1).

The greatest deficit in pulmonary function associated with PM₁₀ exposure was found among children with the *GSTP1* Ile/Ile genotype. For this group, cumulative exposure to PM₁₀ was associated with a decline in FEF_{25-75%} of 23.45% (95% CI: -30.6, -15.56) and a reduction of 13% in FEV₁ (95% CI: -15.21, -10.74) (Figure 3,5; E-table 2).

We found that among *GSTT1* null children, cumulative exposure to PM₁₀ was also associated with a greater reduction in FEV₁, FVC, and FEF_{25-75%} compared to the *GSTT1* non-null genotype (Figure 3-5) (E-table 2).

As for the *GSTM1* genotypes, no differences were found in the associations between PM₁₀ exposure and pulmonary function parameters.

DISCUSSION

In this study, we investigated effect modification by GST (*GSTM1*, *GSTT1*, *GSTP1*) and *TNΦ-a* (*TNΦ-a*-308 and *TNΦ-a*-1031 SNP) gene polymorphisms in the association between particulate matter and pulmonary function in asthmatic children and adolescents. Our results suggested that carriers of the *GSTT1* null allele, as well as the *GSTP1* Ile/Ile genotype, had a greater reduction in pulmonary function associated with exposure to PM₁₀. We also found suggestive evidence for effect modification by *TNΦ-a*-308 and *TNΦ-a*-1031 polymorphisms.

We found an inverse association between PM₁₀ exposure and pulmonary function in most of the subgroup analyses. These results are similar to previous findings, which indicate that acute exposure to air pollutants is associated with a decline in lung function in children³. Identifying genetic and environmental factors that influence lung development in childhood allows the adoption of intervention measures that preserve the lungs. This is particularly important since childhood lung function is a predictor of lung integrity in adulthood and correlates with functional deficits throughout life^{18,19}.

There is growing evidence that oxidative stress mediates the effects of air pollution on respiratory outcomes⁶. GSTs enzymes are generally considered an essential component in the protection against the by-products of oxidative stress²⁰. It has been shown that some GST genes polymorphisms may affect the functioning of the encoded enzymes. The *GSTM1* and *GSTT1* null genotypes are related to a complete absence of the corresponding proteins, while the *GSTP1* Ile/Val and *GSTP1* Val/Val polymorphisms are often associated with reduced enzymatic activity^{20,21}. Such polymorphisms are believed to contribute to susceptibility to the adverse effects of air pollution and individual differences in respiratory outcomes.

In this study, children with the Ile/Ile wild genotypes of *GSTP1* showed a greater reduction in FEV₁, FVC, and FEF_{25-75%}, compared to the variant Ile/Val or Val/Val genotypes. This result contrasts with some previous findings, which suggest that the *GSTP1* Ile/Val or Val/Val genotypes are associated with increased pollutant effects on respiratory parameters among children^{22,23,24} and adults²⁵. Although the allelic variants of *GSTP1* Val decrease the enzymatic activity of glutathione, their presence has also been associated with lower risk of being susceptible to the respiratory effects of air pollutants^{14,26,27}, including a lower decline in lung function^{28,29}.

We found that carriers of the *GSTT1* null allele had a greater reduction in FEV₁, FVC, and FEF_{25-75%} as the exposure to PM₁₀ increased. Few studies have investigated the role of the *GSTT1* genotype in the relationship between exposure to PM₁₀ and lung function. Zhang et al. (2016) found that adults with the *GSTT1* null showed reduced FEV₁ when exposed to diesel combustion particles³⁰. In contrast, Hong et al. (2007) reported no differences in the associations between PM_{2.5} exposure and peak expiratory flow rate among *GSTT1* genotypes in schoolchildren³¹. However, a recent meta-analysis found that the null genotype was associated with increased risk of childhood asthma³². Another study reported that the *GSTT1* null was related to increased inflammation biomarkers among atopic individuals exposed to diesel combustion³³.

We found no evidence of effect modification by the *GSTM1* genotype on the association between PM₁₀ and pulmonary function. Previous reviews reported no consistent evidence regarding the role of *GSTM1* polymorphisms⁹: while some studies report a higher deficit in lung function associated with air pollution among individuals with the *GSTM1* null^{14,28,34,35}, others do not^{21,31}. Moreover, other studies indicate that *GSTM1* may confer greater susceptibility to the respiratory effects of air pollution only in combination with other variant genotypes⁹.

Another related mechanism mediating the effects of air pollution on respiratory outcomes is the induction of a persistent inflammatory state. In the present study, asthmatic children and adolescents with *TNΦ-a* -308 GA or AA variant genotypes had a greater reduction in FEV₁ as the exposure to PM₁₀ increased. The presence of the variant A allele in the *TNΦ-a* -308 SNP can increase the activation of transcription factors by six to seven times, changing the expression of the gene and amplifying the intensity of the inflammatory response to oxidants^{12,13}. Therefore, it is plausible to state that the *TNΦ-a* -308 polymorphism contributes to the reduction of lung function in asthmatics. A higher deficit in pulmonary parameters with an increased exposure to air pollution has also been observed in other studies with different pollutants, including SO₂ and NO³⁶ and ozone³⁷. In contrast, Li et al. (2006) found that children with the *TNΦ-a* -308 GG genotype had lower risk for asthma and wheezing at low levels of ozone exposure (O₃), but this protective effect was reduced at higher O₃ concentrations and even more so in the combination of the variant alleles of the *GSTM1* and *GSTP1* genes³⁸. In other studies, the effect modification by *TNΦ-a* -308 genotypes was observed only in conjunction with the variant genotype of the *GSTP1* gene³⁹.

The conflicting results found in the literature regarding the role of *GST* and *TNΦ-a* genes polymorphisms reinforce the need for further studies. In addition to methodological differences across studies, the heterogeneity of the results could be explained by differences among populations regarding the structure and distribution of effect modifiers, including factors related to air pollution, lung function, and gene expression. It is also worth mentioning that different adjustments for confounding can generate different conclusions regarding causal effect modification or interaction⁴⁰. In the present study, we used causal diagrams to guide the assessment of effect modification, possibly minimizing confounding and collider bias on the estimation of total and cumulative effects.

Our results must be interpreted in light of some limitations. Our reduced sample size might have led to low statistical power, which imposes limitations on the interpretation of null findings and observed differences in pollutant effects across genotypes. In addition, our sample size prevents us from investigating effect modification by possible combinations of polymorphisms and modulation with other environmental factors. Previous findings suggest that complex patterns between genes and pollutants may characterize genetic susceptibility to the respiratory effects of air pollution⁹.

Furthermore, we used data from the nearest background stations to assign a PM₁₀ exposure to each subject. Although this could have reduced measurement error, we cannot rule out the possibility of exposure misclassification.

In conclusion, we found suggestive evidence that genetic variants in *GST* and *TNΦ-a* may modify the association between particulate matter and pulmonary function in asthmatic children and adolescents. Further and larger studies are needed to provide more conclusive evidence regarding the role of *GST* and *TNΦ-a* polymorphisms in the relationship between air pollution and lung function.

Identifying susceptible populations to the respiratory effects of air pollution is essential to the development and implementation of public health policies. Understanding the mechanisms underlying genetic vulnerability is also critical to improve the clinical treatment of asthma and other respiratory diseases.

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FIGURE _1_DAGs.docx available at <https://authorea.com/users/424884/articles/529883-effect-modification-by-gst-and-tnf-%CE%B1-polymorphisms-in-the-association-between-air-pollution-and-lung-function-in-asthmatic-children>

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FIGURE_2_Flow_diagram.docx available at <https://authorea.com/users/424884/articles/529883-effect-modification-by-gst-and-tnf-%CE%B1-polymorphisms-in-the-association-between-air-pollution-and-lung-function-in-asthmatic-children>

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TABLE 1 Study population.docx available at <https://authorea.com/users/424884/articles/529883-effect-modification-by-gst-and-tnf-%CE%B1-polymorphisms-in-the-association-between-air-pollution-and-lung-function-in-asthmatic-children>

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