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GSTM1, GSTP1 and NQO1 polymorphisms and susceptibility to atopy and airway hyperresponsiveness among South African schoolchildren

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Abstract

Gluthathione-S-Transferases (GSTM1 and GSTP1) and Nicotinamide Quinone Oxidoreductase (NQO1) genes play an important role in cellular protection against oxidative stress which has been linked to asthma pathogenesis. We investigated whether common, functional polymorphisms in GSTM1, GSTP1 and NQO1 influences airway hyperreactivity (AHR) and atopy among schoolchildren in South Africa.

Genomic DNA was extracted from 317 primary schoolchildren, aged 9–11 years, from urban, low socioeconomic communities of Durban, South Africa. GSTM1 (null vs present genotype), GSTP1 (Ile105Val; AA→AG+GG) and NQO1 (Pro/187Ser; CC→CT/TT) genotypes were determined using polymerase chain reaction (PCR) methods. Atopy was defined as positive skin prick tests to any of several common allergens. Airway hyperreactivity (AHR) was evaluated by pulmonary function testing before and after methacholine challenge.

Among the children, 30% were GSTM1 null, 65% carried the G allele for GSTP1 and 36% carried the C allele for NQO1. The frequency of GSTM1, GSTP1 and NQO1 variants among our South African sample was similar to frequencies found in similar ethnic groups worldwide. Marked airway reactivity ($PC_{20} \leq 2$ mg/ml) was found in 10.3% of children and approximately 40% of them were atopic. No significant associations were identified for GSTM1 and NQO1 with either AHR or atopy. A significant protective effect against atopy was found among children with one or two copies of the GSTP1 G allele.

Keywords

genetic polymorphism; gluthathione-S-transferases; nicotinamide quinone oxidoreductase; oxidative stress; atopy; AHR

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Introduction

Airway hyperesponsiveness (AHR) has been recognized as a key feature of asthma, characterized by chronic airway inflammation, increased mucus formation, airway wall thickening and smooth muscle dysfunction. AHR often precedes the development of asthma [1,2] but not all subjects with AHR will develop complete expression of asthma [3]. Similarly host atopy is a recognized risk factor for airway inflammation and asthma [4]. Genetic factors may promote or prevent the development of clinically relevant asthma phenotypes among people with AHR and atopy. Genes involved in oxidative stress responses are potential candidate genes for asthma given the role of oxidative stress in airway inflammation and tissue damage. Cells in the lung are protected against oxidative stress by an extensive range of intracellular defenses, including phase II xenobiotic detoxifying enzymes such as Glutathione-S-Transferase enzymes (GSTM1 and GSTP1) and NAD(P)H quinone oxidoreductase 1(NQO1). Polymorphisms in these genes may affect an individual's susceptibility to the oxidant burdens posed by environmental pollutants [5,6].

Approximately 20–50% of individuals lack activity of GSTM1 due to a homozygous gene deletion known as the GSTM1 null genotype [7]. A common polymorphism (Ile105Val) in GSTP1 results in an amino acid change from isoleucine (AA) to valine (GG). The enzyme encoded by GSTP1 utilizes a variety of lipid and DNA products of oxidative stress, and polymorphic variants of this gene are associated with altered catalytic function. Both genes may influence the development and/or severity of respiratory related phenotypes [8]. NQO1 catalyses the detoxification of reactive quinines that can produce reactive oxygen species (ROS) through redox cycling, and a proline to serine change in amino acid (CC to TT, Pro187Ser) results in a loss of enzyme activity [6]. There have been numerous studies documenting associations between these genes implicated in the oxidative stress response and respiratory phenotypes but the data suggests that these associations may not be consistent across ethnic groups owing to differences in intra- and interethnic allele frequencies [9]. To date, there are very limited studies of these genes in relation to risk of asthma and related phenotypes in sub-Saharan Africa. Due to this paucity of data, and as part of a larger study into childhood respiratory outcomes and environmental pollution, we undertook a descriptive study to evaluate the frequencies of GSTM1, GSTP1 and NQO1 polymorphisms in relation to airway hyperreactivity and atopy in a multiethnic sample of South African children.

Methods

Study Population

A total of 317 randomly selected primary schoolchildren (between 9–11 years old) were recruited for this study from schools in Durban, South Africa. The study population consisted of indigenous African children (n=148) (hereafter referred to as "African"), children of Indian (n=67) and European descent (n=20) (hereafter referred to as "Indian" and "White" respectively) and children of mixed ethnicity (n=67). This project was approved by the Ethics Committee of the University of KwaZulu-Natal and the Internal Review Board of the University of Michigan. Informed consent was obtained from all participants and their caregivers.

Respiratory phenotypes

Baseline spirometric assessments and methacholine challenge tests were conducted following American Thoracic Society (ATS) guidelines [10]. The lung function indices of primary interest included forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1). AHR was categorized as marked (PC20 [=dose of methacholine causing a

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20 percent fall in baseline FEV_1] ≤ 4mg/ml), probable (4mg/ml < PC20 ≤ 8mg/ml), borderline/possible (8mg/ml < PC20 ≤ 16mg/ml) and no hyperreactivity (PC20 > 16 mg/ml). Marked, probable and possible AHR were categorized as "any evidence of airway hyperreactivity." Atopy was defined as a positive response to one or more of the following antigens: mixed cockroach, mixed dust mite, mould mix (*Aspergillus, Cladosporium* and *Penicillium*), cat, dog, mouse, rat and grass by skin prick testing, with histamine as a positive control and saline as a negative control. A greater than 3mm difference in mean diameter between allergen and control wheal was considered positive.

Molecular Methods

Genomic DNA was extracted using a PUREGENE DNA isolation kit (cat #D5000). The presence or absence of the GSTM1 gene was determined by using a multiplex PCR method, including the β-globin gene as a positive control [11]. The GSTP1 and NQO1 genotypes were determined by Taqman® SNP Genotyping Assays (Applied Biosystems, Foster City, CA). The NQO1 (rs1800566) and the GSTP1 (rs1695, also known as rs947894) PCR amplifications were performed using the 5′-nuclease assay on Gene-Amp PCR Systems 9700 (Applied Biosystems).

Statistical Analysis

All analyses were conducted using STATA (version 9, College Station, Tx, USA). Initial descriptive analysis was followed by bivariate testing. Differences in genotype frequency between cases and non-cases were assessed by the chi-square test. Associations of genetic variables with AHR and atopy were examined using multivariate logistic regression models using age and gender. Exposure to caregiver smoking was non significant as a covariate.

Results

The mean age of the 317 participating children was 10.1 (SD: ± 1.0) years, with the majority being female (59%), and of African origin (47%). Marked airway reactivity (PC_{20} ≤ 2 mg/ml) was found in 10.3% of children, with an additional 7% of children with evidence of probable AHR. Approximately 40% of the children were atopic. Among African children, 22% showed any evidence of airway reactivity from the methacholine challenge tests, while 30% were atopic (Table 1). The GSTM1 null polymorphism was present in 30% of the participating children, while 65% carried the G allele for GSTP1 and 36% carried the C allele for NQO1 (Table 2). Allele frequencies were assessed only for the GSTP1 and NQO1 polymorphisms, as the method used to analyze GSTM1 did not allow the identification of heterozygotes. The allelic frequencies were 0.42 for the minor G (Val) allele for GSTP1 and 0.24 for the minor T (Ser) allele for NQO1. Frequencies for each gene were consistent with Hardy-Weinberg equilibrium. When stratified by race/ethnicity, the frequencies for GSTP1 G allele ranged from 0.45 for children of mixed ethnicity and 0.52 for Africans to 0.61 for Indians. The frequency of the NQO1 Ser allele was similar in Africans and Indians (0.15 and 0.16 respectively) and 0.36 in children of mixed ethnicity (not shown in tables). Allelic frequencies for whites are presented but not considered to be reliable estimates due to insufficient numbers.

More Indians carried the GSTM1null (38%) and the NQO1 CT+TT (60%) genotypes as compared to Africans and those with mixed race, while Africans showed a relatively higher frequency of the polymorphic GSTP1 AG+GG genotype (79%) (Table 2). Bivariate analyses showed that the distribution of GSTM1null, GSTP1 and NQO1 polymorphisms were not significantly different among participants with and without AHR (Table 3). The proportion of GSTP1 AG+GG was significantly lower in atopic cases as compared to non cases (p=0.02). In Table 3, we present data showing both unadjusted and adjusted logistic

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regression models associations of atopy and AHR and all three genotypes tested among African children. Logistic regression models found no statistically significant difference at the alpha=0.05 level between methacholine testing and any of the three genotypes tested. A significant association between GSTP1 AG+GG and atopy was shown in an unadjusted model and this protective effect was confirmed after adjustment for potential confounders (OR=0.35, 95%CI: 0.13; 0.91). Neither GSTM1 nor NQO1 genotypes were associated with atopy.

Discussion

This is the first published multi-ethnic study describing genetic polymorphisms associated with oxidative stress in Southern Africa. When examining covariate-adjusted associations of these polymorphisms with specific respiratory linked outcomes phenotypes (atopic, and airway hyperresponsiveness) we found that, among African children, the GSTP1AG+GG polymorphism appear to confers some protection against developing atopy.

The polymorphism frequency and allelic distributions seen in our sample were typical of similar populations described previously. A GSTM1 null frequency of 40–60% is common in both Caucasians and Asians, while a relatively lower frequency is usually found in the African populations (16–36%) [9]. Frequencies are comparable to the findings in our study of 40% GSTM1null genotype among White, 38% among Indian and 22% among African children. Fewer studies have been done on GSTP1 and populations from Africa. In our study, the GSTP1 AG+GG frequency was 65%, similar to a previous study among Tunisians [12]. The distribution of the NQO1CC+CT polymorphism was 36%. Frequency of this variant has not been reported previously in an African population, but it was similar to that reported among African Americans [13]. Indians carried the GSTM1 null and NQO1 CT +TT polymorphic variants more frequently than the other groups while black Africans carried the GSTP1 AG+GG genotype more frequently compared to the other race groups.

The prevalence of marked AHR (10%) and atopy (40%) in this South African population was comparable to that found in other studies in South Africa [14,15,16] A study by Steinman and coworkers [15] found a 42.3% prevalence of atopy (defined the same as in this study) and 17% AHR among similar aged multiethnic children. Our finding of a lack of association between GSTM1 and NQO1 with the respiratory linked outcomes tested contrasts with previous results [17,18,19]. This discrepancy between our study and previous reports may be related to differences in study population, smaller sample size, study design or environmental exposures. Although the GSTP1 polymorphism showed no effect on participants exhibiting signs of AHR, the GSTP1 AG+GG polymorphism was significantly associated with a protective effect against developing atopy (OR=0.35, CI: 0.12; 0.96). Using asthma as a respiratory linked phenotype, other authors have similarly found that the GSTP1 Val105/Val105 may protect against developing asthma [4,8], while a case-control study among Tunisian children (5–16 yrs old) in North Africa reported that that the GSTP1 GG genotype showed a 2.33 fold lower risk of asthma than those with the GSTP1AA [12]. In contrast, association studies in other populations worldwide have shown GSTP1 GG to be associated with 3.5 fold higher risk of diagnosed atopic asthma among Turkish adults [17] and with decreased lung function (FEV_1) in a cohort of Californian schoolchildren [18]. Results of association studies with this polymorphism have been inconsistent which may be explained by ethnic and population differences, varied study designs and differing methods of measuring environmental exposures. Since GSTP1 is strongly expressed in the respiratory epithelium and is the dominant GST involved in detoxification of xenobiotics in the lung [17,18], it is postulated that altered GSTP1 activity in bronchial tissue may influence detoxification of airway irritants, inflammation and oxidative stress pathways [17,18,19]. It has been reported that the Val 105 variant has higher catalytic efficiency for polycyclic

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aromatic hydrocarbon diol epoxides but its efficiency for 1-chloro-2,4-dinitrobenzene is lower compared to the Ile105 variant [20]. Thus the effect of GSTP1 polymorphisms on clinical phenotypes may be modulated by the specific pattern of exposure to exogenous chemical agents presenting an oxidative challenge to the individual. Furthermore, GSTP1 may influence the synthesis of eicosanoids, which are critical mediators of the atopic asthmatic response, by modulating ROS levels [4].

Genetic association studies with asthma among African populations are limited and therefore the role of different variants in conferring risk is uncertain. In a comprehensive review on asthma genetics, Ober and Hoffjan [21] examined nearly 500 publications and 79 genes which have been associated with asthma related phenotypes in two or more independent populations. Of these, there are only 25 (3%) publications based on populations of African ancestry (African American, African Caribbean and two populations from the African continent). Unfortunately, most of these studies lack power to detect associations, with sample sizes generally less than 100. Results from studies conducted among mainly Caucasian populations in the northern hemisphere may not be applicable to the situation in Africa due to differences in environmental exposures, ethnicity, socioeconomic status and polymorphism frequencies. Comparisons of African populations show that prevalence of respiratory linked diseases is not similar among different groups further fortifying the argument that these phenotypes appear to be more related to environment and lifestyle than race/ethnicity [22]. However, genetic predisposition to these outcomes, which has been shown to vary across race/ethnicity groups, may be the determining factor in susceptibility.

This study was conducted within a broader investigation into the association of environmental pollution and childhood respiratory outcomes. The latter study included several communities with distinct ethnic character [23]. We believe that the presentation of our findings is important in providing the first description of genetic polymorphisms related to oxidative stress in Southern Africa. Point estimates were suggestive of a protective effect against atopy by GSTP1 AG+GG, but a limitation was the small sample size in this study. We did not include different race groups in the same logistic models due to population stratification effects and the study was not sufficiently powered to investigate differences in genetic susceptibility among different race groups. However, the increased protection conferred by the GSTP1 AG+GG genotype on the development of atopy may have clinical and public health importance since the variant is common in this population and respiratory diseases are a frequent cause of morbidity among children in South Africa.

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Table 1

Demographic and phenotypic characteristics of study population (n=317)

Categories	n (%)
Age, yr (n=317)	10.1 (±1.0) [1]
Sex (n=317)	
Male	131 (41.3)
Female	186 (58.7)
Ethnicity (n=317)	
African	148 (46.7)
Indian descent	82 (25.9)
Mixed ethnicity	67 (21.1)
White	20 (6.3)
Atopic (n=270)	109 (40.4)
AHR (n=271)	
Marked (PC_{20}[2] ≤ 2 mg/ml)	28 (10.3)
Probable (2< PC_{20} ≤8 mg/ml)	19 (7.0)
Possible (8< PC_{20} ≤16 mg/ml)	29 (10.7)
No AHR (PC_{20} > 16 mg/ml)	195 (72.0)

[1] Mean and SD at study entry;

[2] PC_{20} = dose of methacholine causing a 20% fall in baseline FEV_1.

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Table 2

Genotype distribution by race/ethnicity

GENOTYPE (n=317)	RACE/ETHNICITY			
	African N =148 (%)	Indian N=82 (%)	Mixed ethnicity N=67 (%)	White N =20(%)
GSTM1[1]				
Pos (n=221)	115 (77.7)	51 (62.2)	43 (64.2)	12 (60.0)
Null (n=96)	33 (22.3)	31 (37.8)	24 (35.8)	8 (40.0)
GSTP1[2]				
AA (n=105)	29 (21.2)	49 (62.8)	20 (31.2)	7 (41.2)
AG+GG (n=191)	108 (78.8)	29 (37.2)	44 (68.8)	10 (58.8)
NQO1[3]				
CC (n=191)	104 (73.8)	30 (40.0)	43 (68.3)	14 (77.8)
CT+TT (n=106)	37 (26.2)	45 (60.0)	20 (31.8)	4 (22.2)

[1]GSTM1 (positive or null genotype);

[2]GSTP1; A allele codes for isoleucine, G allele codes for valine, AA is the wild type. Ile-Ile (AA), Ile-Val (AG)+Val-Val (GG).

[3]NQO1; C allele codes for Proline, T allele codes for serine, CC is the wild type. Pro (CC), Pro/Ser (CT)+Ser (TT) For GSTP1 and NQO1, we were unable to assign a genotype to about 6% of DNA samples. Since these samples were different for each gene, poor DNA quality could not be a reason for non-amplification.

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Table 3

Association of genotypes with airway hyperreactivity[1] (AHR) and atopy among African children only (n=148)

Genotype		Logistic models			
		AHR		ATOPY	
		OR	95%CI	OR	95%CI
GSTM1					
Positive (n= 115)		1.00		1.00	
Null (n=33)	Unadjusted	0.82	0.27, 2.45	0.95	0.39, 2.31
	Adjusted[2]	0.80	0.26, 2.46	0.92	0.37, 2.25
GSTP1					
AA (n=29)		1.00		1.00	
AG+GG (n=108)	Unadjusted	0.81	0.26, 2.52	**0.34**	**0.14, 0.86** *
	Adjusted[2]	0.72	0.21, 2.45	**0.35**	**0.13, 0.91** *
NQO1					
CC (n=104)		1.00		1.00	
CT+TT (n=37	Unadjusted	1.60	0.60, 4.30	0.68	0.27, 1.71
	Adjusted[2]	1.63	0.59, 4.41	0.70	0.28, 1.76

[1] PC20 < 16 mg/ml

[2] Logistic regression models adjusted for age and gender.

* p-value<0.05