GSTM1, GSTP1 and NQO1 polymorphisms and susceptibility to asthma among South African children

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Gluthathione-S-transferase (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 influence susceptibility to asthma among schoolchildren in South Africa. Genomic deoxyribonucleic acid (DNA) was extracted from 317 primary schoolchildren, aged 9-11 years, from the urban, underprivileged socio-economic communities of Durban. GSTM1 (null vs. present genotype), GSTP1 (Ile105Val; AA →AG+GG) and the NQO1 (Pro/Ser; CC →CT/TT) genotypes were determined using polymerase chain reaction. Among the children, 30% were GSTM1 null, 65% carried the G allele for GSTP1, and 36% carried the C allele for NQO1.There was a high prevalence of asthma of any severity (46.1%), with 20.4% reporting persistent asthma. The GSTP1 AG+GG polymorphic genotype was significantly associated with persistent asthma (adjusted OR = 3.98; CI = 1.39, 11.36, p-value = 0.01). Neither the GSTM1, nor the NQO1, genotype was a significant predictor of persistent asthma. Therefore, the GSTP1 A/G variant may modulate the risk of persistent asthma among our sample.

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Introduction

Asthma is a chronic disease, characterised by reversible airflow obstruction and airway inflammation that affects as many as 300 million people worldwide, with increasing morbidity and mortality, especially in developing countries.[1] Asthma presents a substantial public health burden, particularly for children, both in the number of people affected by the disease, and the related morbidity and cost. In Africa, reported prevalence has ranged from 5.1% in Nigeria, to 26.5% in South Africa.[2] The increasing prevalence worldwide has been attributed to several factors, including changing environmental, dietary and in utero factors. Despite these external influences, asthma tends to aggregate within families, suggesting the importance of genetic susceptibility. Current studies indicate that many regions of the human genome are associated with various asthmatic phenotypes.[3] Genes involved in oxidative stress responses are potential candidate genes for asthma, given the role of oxidative stress in airway inflammation. 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 nicotinamide quinone oxidoreductase 1(NQO1) genes.[4,5] Polymorphisms in these genes may affect an individual's susceptibility to the oxidant burdens posed by environmental pollutants.

Approximately 20-50% of individuals lack activity of GSTM1, due to a homozygous gene deletion known as the GSTM1null genotype.[6] A common polymorphism (Ile105Val) in GSTP1 results in an amino acid change from isoleucine (AA) to valine (GG). Both genes may influence the development and severity of respiratory disease-related phenotype.[7] NQO1 catalyses the detoxification of reactive quinines that can produce reactive oxygen species (ROS) through redox cycling. A proline to serine change in amino acid (CC to TT, Pro187Ser) results in a loss of enzyme activity.[5] There have been numerous studies documenting associations between these genes implicated in the oxidative stress response, and various asthma phenotypes, but the data suggest that associations between genetic polymorphisms and asthma may not be extrapolated from one ethnic group to another, based on differences in intra- and interethnic allele frequencies.[8] To date, two studies have been published from North Africa[9,10] specifically in relation to GSTs

and asthma, and to the best of our knowledge, our study is the only sub-Saharan work in this field. In view of the limited data in the African context, we undertook a descriptive study to evaluate the frequencies of GSTM1, GSTP1, and NQO1 polymorphisms and susceptibility to asthma in a multi-ethnic sample of South African children.

Method

Study population

A sample of children between 9-11 years old from communities in south and north Durban was randomly selected from seven primary schools. Residential areas from the Durban South area were Merebank, Wentworth/Austerville, Bluff and Lamontville. Northern residential areas within the metropolitan boundaries of Newlands East, Newlands West and KwaMashu were selected. At each of the seven schools, two fourth-grade classes were randomly prioritised as Classroom 1 and Classroom 2. Students in these classrooms were asked to complete a screening questionnaire, which included questions relating to diagnosed asthma, frequency of symptoms, and details of household adult membership. The final sample included 317 children, who represented a random, population-based sample, which permitted description of community-based prevalence of disease outcomes among children. 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), hereafter referred to as "coloured." 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

Interviews were conducted with participants and their caregivers. Questions addressed demographics, and assessment of presence and severity of respiratory, and other, relevant symptoms, using standardised validated questions from sources including the British Medical Research Council and the American Thoracic Society (ATS).[11] Asthma severity was categorised in two ways, as asthma of any severity, and persistent asthma. A child was considered to have asthma of any severity if any of the following were true: three or more non-exercise-related symptoms, e.g. coughing, wheezing or chest tightness), exercise-induced wheezing or coughing, reported at a frequency of three, or more, times during the previous year; doctor-diagnosed asthma, reactive airway disease and asthmatic bronchitis, or doctor-prescribed medication taken in the previous year. A child was considered to have persistent asthma if, firstly, the child met the diagnostic criteria for asthma of any severity, and secondly, if any of the following were true: any daytime symptoms (coughing, wheezing, exercise-induced coughing, wheezing and chest tightness) were reported as being present more than two times per week; sleep disturbances due to coughing, wheezing, shortness of breath or chest tightness, reported more than two times per month; and/or daily use of doctor-prescribed medication.[12]

Molecular methods

Genomic deoxyribonucleic acid was extracted using a Puregene DNA Isolation Kit (cat #D5000). The presence or absence of the GSTM1 gene was determined by using a multiplex polymerase chain reaction (PCR) method, including the b-globin gene as a positive control.[13] 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 carried out using Stata® (version 9, College Station, Tx, USA). Initial descriptive analysis was followed by bivariate testing. GSTM1 was dichotomised into the null genotype and the present genotype, whereas the GSTP1 and NQO1 polymorphisms were categorised into two groups, based on the absence or presence of the polymorphic allele (wild-type homozygous, heterozygote and homozygous variant). The heterozygote and homozygote variants were combined in data analysis models. Associations of genotype with asthma were examined using multivariate logistic regression models, using race, age, gender, and exposure to environmental tobacco smoke, as covariates. Race was included as African, compared to “other”, which was inclusive of Indian, coloured and white.

Results

The mean age of the 317 participating children was 10.1 [standard deviation (SD): ± 1.0] years, with the majority being female (59%), and of African origin (47%). Approximately 58% were exposed to environmental tobacco smoke through a caregiver, or a live-in family member smoking (Table I). 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 II). A varied distribution of the GSTP1 AG/GG genotype was evident among different race groups with the African and coloured populations having the highest frequencies (78.6% and 69.0% respectively). The GSTM1 null frequency varied among race groups with the lowest frequency (21%) recorded for the African population. Frequencies for each gene achieved Hardy-Weinberg equilibrium.

More children of Indian origin carried the GSTM1 null (38%) and the NQO1 CT+TT (60%) genotypes, compared to the other race groups, while Africans had a relatively higher frequency of the polymorphic GSTP1 AG+GG genotype (79%). Almost 26% were homozygous for the polymorphic G allele (Table II). There

was a high prevalence of asthma of any severity (46.1%), with 20.4% reporting persistent asthma. Of the children with reported symptoms of asthma of any severity, 36 (27%) carried the GSTM1 null genotype, 79 (65%) the GSTP1 AG+GG genotype, and 49 (39%) the NQO1 CT+TT genotype (Table III). Among children reporting persistent asthma, 18 (31%) carried the GSTM1 null genotype, 40 (75%) the GSTP1 AG+GG genotype, while 14 (26%) carried the NQO1 CT+TT genotype. The GSTP1 AG+GG genotype was significantly associated with persistent asthma (adjusted OR = 3.98; CI:139, 11.36; p-value = 0.01) among African children, compared to other race groups. Neither the GSTM1 nor the NQO1 genotypes were significant predictors of persistent asthma.

Table I: Demographic and phenotypic and genotypic characteristics of study population

Categories	n (%)
Age, years (n = 317)	10.1 (0.96)*
Sex (n = 317)	
Male	131 (41.3)
Female	186 (58.7)
Race (n =317)	
African	148 (46.7)
Indian	82 (25.9)
Coloured	67 (21.1)
White	20 (6.3)
Exposure to environmental tobacco smoke (n = 256)**	149 (58.2)
Prevalence of respiratory outcomes (n = 284)* **	
Any asthma	131 (46.1)
Persistent asthma (n = 284)	58 (20.4)

Table adapted from Reddy, Naidoo, Robins, et al, 2010.[14]

* Mean and standard deviation at study entry
** Environmental tobacco smoke includes caregiver smoking and presence of any smoking in the household. Environmental tobacco smoke data was available for 256 participants only
*** Asthma data complete for 284 participants

Discussion

In this, the first multi-ethnic study describing genetic polymorphisms associated with oxidative stress in southern Africa, we demonstrated that the GSTP1 AG+GG polymorphism is likely to be associated with specific adverse respiratory outcomes. Our results support the hypothesis that individual capacity to mount an effective protective response against oxidative stress, as determined by a polymorphism on GSTP1, contributes to persistent childhood asthma.

The prevalence of any grade of asthma (46.1%), based on symptoms, was higher than the 20.3% reported for asthma symptoms in 13- to 14-year-old children in South Africa.[2] However, a preliminary study at a primary school in south Durban, investigating the relationship between asthma and ambient pollutant exposure, revealed a prevalence of 52% of any grade among 9- to 11-year-old children,[15] similar to

Table II: Genotype distribution stratified by race

Genotype (n = 317)	Race			
	African (n =148)%	Indian (n = 82)%	Coloured (n = 67)%	White (n = 20)%
GSTM1				
Present	115 (77.7)	51 (62.2)	43(64.2)	12 (60.0)
Null	33 (22.3)	31 (37.8)	24 (35.8)	8 (40.0)
GSTP1				
AA	29 (21.2)	49 (62.8)	20 (31.2)	7 (41.2)
AG	73 (53.3)	24 (30.8)	30 (46.9)	10 (58.8)
GG	35 (25.6)	5 (6.4)	14 (21.9)	0 (0.0)
AG+GG	108 (78.8)	29 (37.2)	44 (68.8)	10 (58.8)
NQO1				
CC	104 (73.8)	30 (40.0)	43 (68.3)	14 (77.8)
CT	33 (23.4)	36 (48.0)	20 (31.8)	4 (22.2)
TT	4 (2.8)	9 (12.0)	0 (0.0)	0 (0.0)
CT+TT	37 (26.2)	45 (60.0)	20 (31.8)	4 (22.2)

Table III: Odds ratios of asthma phenotype by GSTM1, GSTP1 and NQO1 genotypes

	Any asthma			Persistent asthma		
	Present n (%)	OR	CI	Present n (%)	OR	CI
GSTM1						
Positive (n = 200)	95 (47.5)	1.00	0.44-1.49	40 (20.0)	1.00	
Null (n = 84)	36 (42.8)	0.81		18 (21.4)	1.93	0.88-4.24
GSTP1						
AA (n = 95)	42 (44.2)	1.00	0.68-2.33	13 (13.6)	1.00	
AG+GG (n = 171)	79 (46.1)	1.26		40 (23.4)	3.98	1.39-11.36*
NQO1						
CC (n = 171)	75 (43.9)	1.00	0.73-2.41	40 (23.4)	1.00	
CT+TT (n = 94)	49 (52.1)	1.33		14 (14.9)	0.58	0.24-1.42

Logistic regression models adjusted for age, race, gender, and environmental exposure to tobacco smoke

* p-value = 0.01

the prevalence found in the current study. The prevalence of persistent asthma (20%) was comparable to that found in other South African studies.[2,16,17] The International Study of Asthma and Allergies in Childhood (ISAAC) found a 15% prevalence of asthma symptoms in a South African study of 13- to 14-year-old children. However, the prevalence of asthma found in this study is still much higher than the mean prevalence of asthma in southern Africa (8.1%), as determined by the Global Strategy for Asthma Management and Prevention (GINA) report.[18]

According to the literature on metabolic gene frequencies in control populations, a GSTM1 null frequency of 40-60% is common in both Caucasians and Asians, while a lower frequency is usually found in the African populations (16-36%).[8,18] Our results on GSTM1 among Africans concur with other African population studies, which have indicated a relatively low genotypic frequency of the null genotype (22%) (Table II). This was similar to the 24% GSTM1 null genotype found in Zimbabweans, and 23% and 21% GSTM1 null found in South African Vendas and Xhosas, respectively.[19] Fewer studies have been carried out with GSTP1 among similar populations. One study found a 75.6% frequency of GSTP1 AA, and 24.4% GSTP1 AG+GG in a Asian population,[20] while the frequency of this variant in a South African Xhosa population was 22% for GSTP1AA, and 78% for GSTP1 AG+GG.[19] This was similar for Africans in our study. Twenty-one per cent carried the GSTP1AA, and 79%, the GSTP1 AG+GG genotype. Other studies have shown that the frequency of the NQO1 TT homozygous genotype varies across ethnic groups, 4% in Caucasians, 5% in African Americans, and 22% in Asians.[21] In this study, we found the frequency of the NQO1 TT polymorphism was 4.3% ,and the combined heterozygote and homozygote (NQO1CC+CT) was 36%. African and coloured children presented with the lowest GSTM1 null frequencies, 22% and 36% respectively, which are much lower than that of other populations reported in literature.[8] Similar frequencies in African and coloured populations may be due to their closely linked ancestries.

Our findings of excess risk of the association of variants of GSTM1, GSTP1 and NQO1 with respiratory-related phenotypes are supported by other studies.[21-23] However, with the exception of the GSTP1 AG+GG variant and persistent asthma, no significant associations were found in our study. This may be related to a small sample size, and the multi-ethnic composition of our sample. Asthma diagnosis based on reported symptoms is an additional limitation. GSTP1 is strongly expressed in the respiratory epithelium, and is the dominant GST involved in detoxification of xenobiotics in the lung,[21,22] which could account for the GSTP1 variant having a larger effect on persistent asthma, than the other two genes tested. It is postulated that lower GSTP1 activity in bronchial tissue may result in decreased detoxification of airway irritants, enhanced inflammation, and oxidative stress, causing sustained airway wall thickening and smooth muscle thickening.[24]

Results in our study concur with association studies in different populations worldwide, in which the GSTP1 GG variant has been reported to increase the risk of asthma,[21] and to increase susceptibility to the effects of ozone on respiratory difficulties in children.[25] However, this allele, although associated with reduced glutathione activity, was also reported to be protective for asthma and airway responsiveness.[7,9,20] This was evident from a study in Tunisia, in which children with the GSTP1 GG genotype had a 2.33-fold lower risk of acquiring asthma, than those with the GSTP1 AA;[9] and one in Egypt,[10] in which the frequency of GSTP1 AA was significantly increased in asthmatics, compared to controls. Other studies have reported no association between the GSTP1 polymorphism and asthma.[26-28] A meta-analysis on GSTP1 and asthma indicated no clear evidence of a small study bias, although heterogeneity in the study results was large, and limited the interpretability of pooled estimates. However, the meta-analysis revealed a potential role of ethnicity, with the protective effect of the GSTP1GG allele being very strong in the two studies from North Africa.[29]

Genetic association studies with asthma among African populations are limited. Therefore, the role of different variants in conferring risk is uncertain. In a comprehensive review on asthma genetics, Ober and Hoffjan[3] examined nearly 500 publications and 79 genes which have been associated with asthma-related phenotypes in two, or more, independent populations. Of these, only 25 (3%) of publications are based on populations of African ancestry (African American, African Caribbean, and two populations from North Africa). 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, socio-economic status, and gene frequencies. Comparisons of African populations show that asthma prevalence is not similar among different groups, further strengthening the argument that asthma appears to have more of a link to the environment and lifestyle, than race.[30] However, genetic predisposition to these outcomes, which has been shown to be variable among different race groups, may be the determining factor with regard to susceptibility.

Despite the limitation of a small sample size, this study indicated that the GSTP1 AG+GG variant, which is involved in an individual's susceptibility to oxidative stress, may increase the risk of persistent asthma among our sample. The increased susceptibility conferred by the GSTP1 variant may have clinical, and public health, importance, since the variant is common in this population, and respiratory diseases are frequent causes of morbidity among children in South Africa. Future research in this population should focus on environmental factors which may potentially interact with GST and NQO genes in modifying asthma risk.

Declaration

All the authors of this manuscript declare no commercial or other association that might pose a conflict of interest.

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