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# *NFE2L2* gene variants and susceptibility to childhood-onset asthma

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ABSTRACT

**Introduction.** Environmental factors causing oxidative stress are known to be associated with asthma morbidity. The antioxidative gene *NFE2L2* has been implicated in asthma development in mice models. In humans, the SNPs -617C/A and -653G/A, located at the promoter region of *NFE2L2* gene, have been found associated with the susceptibility to develop diverse chronic-degenerative diseases. **Objective.** To determine if there is association of the -617C/A and -653G/A *NFE2L2* SNPs and childhood-onset asthma in a Mexican population. **Materials and methods.** In a case-control study 242 unrelated patients with diagnosis of asthma and 358 ethnically- and sex-matched healthy individuals were included. The -617C/A and -653G/A *NFE2L2* genotyping was carried out using the TaqMan allelic discrimination assay. **Results.** The risk allele of both polymorphisms showed a high frequency in our sample (-617A: 24% and -653A: 40%), similarly to those previously reported in Asiatic populations (-617A: 24-29% and -653A: 42-52%; $p > 0.05$). In contrast, the -617A allele frequency was higher than that reported in a European-African admixed population (10%, $p < 0.001$). The allelic and genotypic frequencies from both polymorphisms showed no significant differences among cases and controls in female and male samples. Likewise, haplotype analysis found no association between *NFE2L2* gene variants and the disease. **Conclusions.** Despite the experimental evidence suggesting that *NFE2L2* gene is involved in asthma pathogenesis, the -617C/A and -653G/A SNPs were not associated with childhood-onset asthma.

**Key words.** Childhood-onset asthma. *NFE2L2* gene. Polymorphisms.

*NFE2L2 variantes en la susceptibilidad por asma infantil*

*RESUMEN*

***Introducción.*** *Factores ambientales que causan estrés oxidativo se han asociado con asma. El gen antioxidante NFE2L2 se ha implicado en el desarrollo de esta patología en diversos modelos murinos. Los polimorfismos -617C/A y -653G/A, localizados en la región promotora de NFE2L2, se asocian con la susceptibilidad a desarrollar diferentes enfermedades crónico-degenerativas.* ***Objetivo.*** *Determinar la posible asociación de los polimorfismos -617C/A y -653G/A en NFE2L2 con la susceptibilidad a desarrollar asma infantil en población mexicana.* ***Material y métodos.*** *Diseño de casos y controles, 242 individuos no relacionados, con diagnóstico clínico de asma y 358 donadores sanos, pareados por género y ancestría. La genotipificación de los SNPs -617C/A y -653G/A del gen NFE2L2 se realizó mediante la técnica de discriminación alélica 5' exonucleasa TaqMan.* ***Resultados.*** *Los alelos de riesgo de ambos polimorfismos presentaron una alta frecuencia en nuestra población (-617A: 24% y -653A: 40%), la cual fue similar a la reportada en poblaciones asiáticas (-617A: 24-29% y -653A: 42-52%; $p > 0.05$). La frecuencia del alelo -617A fue más alta que la reportada en una población de origen europeo-africano (10%, $p < 0.001$). Tanto las frecuencias alélicas y genotípicas de ambos polimorfismos como los haplotipos no mostraron diferencias significativas entre los casos y los controles.* ***Conclusiones.*** *A pesar de que existen evidencias experimentales que sugieren la participación del gen NFE2L2 en la patogénesis del asma, en este estudio los polimorfismos -617C/A y -653G/A no mostraron asociación con asma infantil.*

***Palabras clave.*** *Asma. Estrés oxidativo. NFE2L2. Polimorfismos.*

## INTRODUCTION

Asthma is a chronic lung disease characterized by reversible airway obstruction, inflammation, increased airway responsiveness and excessive mucus production, which vary in severity and frequency from person to person. This entity is the most common chronic disease among children which prevalence is increasing in developing countries.[1] Environmental factors causing oxidative stress are known to be associated with asthma morbidity.[2,3] Hence, defective critical host factors that protect the lungs against oxidative stress may either determine asthma susceptibility or modifiers of disease severity. The nuclear erythroid-derived 2-related factor 2 gene (*NFE2L2*) encodes to a pivotal molecule in cellular defence against oxidative stress and xenobiotics, the transcription factor Nrf2.[4] This transcription factor regulates the expression of several genes encoding important anti-oxidant proteins such as, heme-oxigenase 1 (HO-1) and NAD(P)H: quinone oxidoreductase 1 (*NQO1*), as well as several proteins involved in detoxification and excretion, for instance glutathion-S transferase (GST), glutamate-cystein ligase (GCLM) and multidrug resistance proteins (MRPs).[5,6] It has been shown that Nrf2 disruption enhances inmunoreactivity and airway inflammation after exposure to certain environmental factors.[7,8] On the other hand, induction of Nrf2 activity confers cellular protection against different environmental asthma-related factors.[9] Moreover, *NFE2L2* knockout mice exhibit increased susceptibility to allergen-induced asthma (CD1:ICR).[10]

Recently, the *NFE2L2* single nucleotide polymorphisms (SNPs) -617C/A and -653G/A, were shown to reduce promoter activity *in vitro.*[11] Besides, these SNPs have been associated with different human diseases.[12-15] Interestingly, functional SNPs located in antioxidant (*NQO1*) and detoxificant genes (*GSTT1, GSTM1* and *GSTP1*) have been associated with individual differences in the susceptibility to asthma development.[16,17] However, despite the strong experimental evidence highlighting the possible involvement of *NFE2L2* in asthma susceptibility, to date there are no studies on the possible relationship between polymorphisms in the *NFE2L2* gene and asthma.

## OBJECTIVE

The aim of this study was to determine whether the -617C/A and -653G/A polymorphisms were associated with childhood-onset asthma in a Mexican population.

## MATERIAL AND METHODS

### Patients and controls

A total of 600 unrelated Mexican individuals were recruited in the study, including 242 patients diagnosed with childhood-onset asthma (38% females and 62% males) and 358 sex- and age-matched healthy controls. All patients were < 17 years of age at the time of enrolment. Patients were recruited from four tertiary care hospitals located in Mexico City. Diagnosis of asthma was made according to American Thoracic Society and Global Initiative for Asthma (GINA) criteria.[18] Population stratification was evaluated with a panel of 10 informative ancestry markers, as previously reported.[19] Controls were enrolled from a blood bank in Mexico City and were > 18 years of age. All the control individuals lack symptoms or history of asthma, allergy or other pulmonary diseases, and no first-degree relatives with a history of asthma. The protocol was approving by local ethics and research committees and an informed written consent was obtained from all participants.

### Genotyping

Genomic DNA was isolated from 10 mL of peripheral blood, using the QIAamp DNA Blood Maxi kit (Qiagen, Valencia CA). Determination of -617C/A and -653G/A genotypes was performed by the TaqMan allelic discrimination assay (Applied Biosystems, Foster City, CA). To validate TaqMan genotyping, 100 random samples were analyzed by direct sequencing. Primers and probes used in this study are available on request.

### Statistical analysis

Significant differences between cases and controls were determined using $\chi^2$ analysis. Odds ratio (OR) was calculated using allele frequencies in a 95% confidence interval (95% CI). Statistical calculations and Hardy–Weinberg equilibrium were performed using StatCalc software (Epi Info 2005 v3.3.2; Centers of Disease Control and Prevention, Atlanta, GA, USA) and the FINETTI program (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl), respectively. Haploview v3.2 was used to determinate haplotype frequencies and associations for each possible combination of haplotypes. The statistical power for the study was estimated using QUANTO software (version 1.2; http://hydra.usc.edu/GxE/) and it reached > 80% under dominant model.

## RESULTS

The patients ranged in age from 5 to 17 years, fulfilled the GINA criteria for asthma and the mean (± SD) age at onset was 10.84 (SD ± 3.1) years old. After genotyping *NFE2L2* polymorphisms, frequencies of -617C/A and -653G/A SNPs were found in Hardy–Weinberg equilibrium, in both cases and controls ($p > 0.05$). The -617A and -653A risk alleles were found with a frequency of 24 and 40%, respectively, in healthy population. These allele frequencies were similar to those previously documented in several Japanese populations, where -617A and -653A shown a range from 24 to 29% and from 42 to 52%, respectively ($p > 0.05$).[12,13,20] In contrast, the frequency of -617A allele obtained in our sample was significantly different to that reported in an European-African admixed population (10%; $p < 0.001$).[11]

When we compared allelic and genotypic frequencies of -617C/A and -653G/A no significant differences between cases and controls were observed (Table 1). Stratification by gender failed to show association among *NFE2L2* SNPs and asthma either in female or male populations (Table 2).

Haplotype analysis from -617C/A and -653G/A *NFE2L2* SNPs, displayed three different haplotypes with a frequency > 1%. The most frequent haplotype was composed for both ancestral alleles (CG: 59%), followed by the haplotype harbouring the minor alleles (AA: 24%) and the haplotype containing a combination of an ancestral and a risk allele (CA: 17%). Haplotype frequencies showed no significant differences between cases and controls (Table 1), even when data were stratified by gender (data no shown).

## DISCUSSION

Asthma is a chronic inflammatory airway disease that is characterized by systemic and chronic localized inflammation and oxidative stress. Sources of oxidative stress could arise from either environmental insults or increased burden of reactive oxygen species (ROS) released from inflammatory cells. Increased levels of ROS may play a role in enhancing the inflammatory response in asthma. *NFE2L2,* the master gene in the cellular protection against oxidative stress, plays an essential role in the pathogenesis of several chronic diseases. Although several studies have highlighted the asso-

**Table** 1. Association of *NFE2L2* -653G/A and -617C/A SNPs in childhood-onset asthma Mexican patients.

| Genotype | Case N = 242 | Control N = 358 | | OR | CI[95%] | P |
|---|---|---|---|---|---|---|
| -653G/A | | | | | | |
| GG | 0.37 | 0.36 | | | | |
| GA | 0.44 | 0.47 | GG *vs.* GA | 0.91 | 0.630-1.303 | 0.594 |
| AA | 0.19 | 0.17 | GG *vs.* AA | 1.14 | 0.717-1.815 | 0.579 |
| Alleles | | | | | | |
| G | 0.58 | 0.60 | | | | |
| A | 0.42 | 0.40 | | 1.04 | 0.825-1.319 | 0.722 |
| -617C/A | | | | | | |
| CC | 0.59 | 0.58 | | | | |
| CA | 0.35 | 0.37 | CC *vs.* CA | 0.89 | 0.634-1 268 | 0.537 |
| AA | 0.06 | 0.05 | CC *vs.* AA | 1.11 | 0.536-2.309 | 0.774 |
| Alleles | | | | | | |
| C | 0.77 | 0.76 | | | | |
| A | 0.23 | 0 24 | | 0.96 | 0.736-1 270 | 0.809 |
| Haplotypes | | | | | | |
| GC | 0.58 | 0.59 | | 0.94 | 0.69-1.28 | 0.621 |
| AA | 0.23 | 0 24 | | 0.95 | 0.69-1.54 | 0.693 |
| AC | 0.19 | 0.17 | | 1.15 | 0.74-1.51 | 0.352 |

OR: Odds ratios. CI: Confidence interval. P value corrected by Bonferroni method.

Table 2. Association of *NFE2L2* -653G/A and -617C/A SNPs in childhood-onset asthma by gender.

| SNP | Genotype | Female | | Male | | | Female | | Male | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Case N = 92 | Control N = 139 | Case N = 150 | Control N = 219 | | OR | P | OR | P |
| -653G/A | GG | 0.39 | 0.39 | 0.35 | 0.34 | | | | | |
| | GA | 0.44 | 0.45 | 0.43 | 0.48 | GG *vs.* GA | 0.97 | 0.911 | 1.47 | 0.254 |
| | AA | 0.17 | 0.16 | 0.22 | 0.18 | GG *vs.* AA | 1.04 | 0.913 | 0.74 | 0.512 |
| | *Alleles* | | | | | | | | | |
| | G | 0.61 | 0.61 | 0.56 | 0.58 | | | | | |
| | A | 0.39 | 0.39 | 0.44 | 0.42 | | 1.01 | 0.951 | 0 94 | 0.78 |
| -617C/A | CC | 0.65 | 0.58 | 0.56 | 0.57 | | | | | |
| | CA | 0.30 | 0.37 | 0.37 | 0.37 | CC *vs.* CA | 0.72 | 0.874 | 1.32 | 0.386 |
| | AA | 0.05 | 0.05 | 0.07 | 0.06 | CC *vs.* AA | 0.90 | 0.270 | 0.32 | 0.140 |
| | *Alleles* | | | | | | | | | |
| | C | 0.80 | 0.77 | 0.75 | 0.76 | | | | | |
| | A | 0.20 | 0.23 | 0.25 | 0 24 | | 0.81 | 0.377 | 0.89 | 0.651 |

OR: Odds ratios. P value corrected by Bonferroni method.

ciation of functional SNPs in *NFE2L2* with diverse chronic-degenerative diseases,[12-15] to our knowledge no studies about association of *NFE2L2* gene variants and asthma have been performed. Hence, the aim of this investigation was to test whether *NFE2L2* is associated with childhood-onset asthma in Mexican population.

*NFE2L2* -617A and -653A allele frequencies obtained from Mexican controls were in concordance with those described in several studies of Japanese population.[12,13,20] This similarity between Mexican and Asian has been repeatedly described to other SNPs.[21,22] These findings could be explained because of the Amerindian ancestry of Mexican population, which has been related with the Asiatic populations.[23] On the other hand, the frequency of -617A risk allele was significantly higher in Mexican-Mestizo than that reported in populations where is well known that their ancestral influence is low in Mexican population (24 *vs.* 12%),[11] such as non-Hispanic Caucasian and African populations.[24,25]

Diverse experimental evidence suggested that polymorphisms affecting *NFE2L2* activity might have fundamental importance in asthma development. Accordingly, polymorphisms in antioxidant genes such as *NQO1* and detoxificant genes like *GSTM, GSTT* and *GSTPI,* have been associated to asthma susceptibility and severity in various human populations.[26,27] Despite the experimental evidence suggesting that *NFE2L2* gene is involved in asthma pathogenesis, the -653G/A and -617C/A SNPs were not associated with childhood-onset asthma in Mexican population, although we cannot discard association with asthma severity. Further studies analyzing different asthma phenotypes [eg, severe persistent asthma, bronchial hyperresponsiveness and increased total serum immunoglobulin (Ig) E levels] in larger samples are needed to evaluate whether might be an association between genetic variants on *NFE2L2* and asthma.

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