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Short communication

# Polymorphisms in inflammasome genes and risk of asthma in Brazilian children

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ABSTRACT

Considering its role in inflammation and recently described "alternative" roles in epithelial homeostasis and Th1/Th2 balance, we hypothesize that inflammasome genetics could contribute to the development of asthma. Selected functional polymorphisms in inflammasome genes are evaluated in a cohort of asthmatic children and their families.

Gain-of-function *NLRP1* variants rs11651270, rs12150220 and rs2670660 resulted significantly associated to asthma in trios (TDT) analysis; and rs11651270 and rs2670660 also with asthma severity and total IgE level in asthmatic children. NLRP1 activators in humans are still unknown, however we hypothesized that individuals with gain-of-function SNPs in *NLRP1* could be more prone in activating inflammasome in the presence of asthma-related cell stressors (i.e. ER stress or ROS), and this activation contribute to exacerbate inflammatory response and asthma development.

Gain-of-function *IL1A* rs17561 resulted significantly associated with a reduced pulmonary capacity in asthmatic children. *IL18* rs5744256 which lead to lower serum level of IL-18 appeared to be associated to a worse response to bronchodilators.

Concluding, this work provides evidences about the contribution of inflammasome genetics in the development of paediatric asthma, both considering its inflammatory role in alveolar macrophages (i.e.: *NLRP1*) or its homeostatic role in lung epithelial cells (i.e.: *IL1A, IL18)*.

## 1. Introduction

Although asthma have a clear familial component, asthma and asthma-related traits are nowadays known as typical complex disease. Several efforts have been made to elucidate its multifactorial pathogenesis, however the entire genetic component as well as the elucidation of heterogeneity in clinical presentation are still missing.

Genome wide association studies (GWAS) indicate that genes codifying for molecules involved in innate immune response and inflammation, such as Toll-like receptors (TLRs; i.e.: *TLR2*, *TLR4*), pro-inflammatory cytokines (i.e.: *TNF*, *IL18*) and eicosanoids (i.e.: *PTGE2R*), are strong contributing factors in the development of asthma. Other genes frequently associated with asthma are related to the Th2 polarization, epithelial cells homeostasis, and lung function and airways remodelling (Bonnelykke et al., 2015).

The inflammasome is a cytoplasmic complex which senses microbes and cellular damage through a pattern recognition receptor (NLR or ALR), activate caspase-1 and induces the release of the pro-inflammatory cytokines IL-1ß and IL-18 (Rathinam and Fitzgerald, 2016). This activation lead to an inflammatory response that is critical for the resolution of infections and repair of tissue damage. Due to its role, inflammasome is involved in chronic inflammation and represents a main contributor of several multifactorial diseases, such as atherosclerosis, obesity and metabolic disease, cancer, neurodegenerative disorders, autoimmunity (Rathinam and Fitzgerald, 2016). Recently NLRP3 inflammasome has also been associated with eicosanoids production (Rathinam and Fitzgerald, 2016). On the other hand, recent studies indicate that inflammasome components also participate in non-inflammatory processes such as epithelial homeostasis (Rathinam and Fitzgerald, 2016) and Th2 differentiation (Rathinam and Fitzgerald, 2016).

Taking in account the well-known inflammatory role of inflammasome molecules together with their contribution to epithelial homeostasis and Th1/Th2 balance, we hypothesize that it could participate

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to the development of asthma.

Hitomi *et coll.* (Hitomi et al., 2009) have shown that a gain-of-function single nucleotide polymorphism (SNP) in 3′UTR of NLRP3 gene (rs10754558) represents a protective factor against the development of food-induced anaphylaxis, suggesting that inflammasome genetics could have a role in hypersensitivity. Since this study (Hitomi et al., 2009), to our knowledge, inflammasome genetics have not yet been evaluated in other hypersensitivity diseases. For this reason, and considering recent findings about "alternative" roles of inflammasome molecules, selected SNPs in inflammasome genes were analysed in a group of Brazilian families with asthma.

## 2. Materials and methods

### 2.1. Asthma cohort

121 children with asthma were recruited in the period 2003–2010 at the Ambulatory of Allergy, Medical School of Federal University of São Paulo (São Paulo, SP, Brazil), after written informed consent approved by the Committee of Ethics on Research. For 74 of them, parents have also been included in the study (trios). Children (male/female: 69/52; mean age: 10.6 ± 3.5 years) received a diagnostic of asthma based on the Global Strategy for Asthma Management and Prevention (Global Initiative for Asthma/GINA, 2002; www.ginasthma.org). For analysis purpose, patients were grouped based on clinical presentation in mild (intermittent and persistent mild asthma; n = 68) and severe asthma (moderate and severe persistent asthma; n = 53); with a good or bad response to bronchodilators (forced expiratory volume in one second, FEV1, ≥7% or < 7% respectively). Main individuals' clinical and laboratorial characteristics are reported in Table 1.

### 2.2. SNPs selection and genotyping

7 SNPs in the inflammasome genes *NLRP1* (rs12150220, rs11651270, rs2670660), *NLRP3* (rs10754558), *IL1A* (rs17561), *IL1 B* (rs1143634), *IL18* (rs5744256) were selected based on functional effect, minor allele frequency (MAF) and/or previously reported association with human disorders. SNPs genotyping was performed using commercially available allele-specific TaqMan assays (*Applied Biosystems/AB, Thermofisher Scientific*) using Quant Studio Real-Time platform (AB).

### 2.3. Association analysis

The analysis of SNPs distribution was executed in the asthma cohort (multivariate analysis) as well as within families (trio analysis). The Haploview software was used for trio analysis and linkage disequilibrium (LD) test. Multivariate analysis based on general linear model (GLM) was executed using the R-project package "SNP-assoc".

## 3. Results and discussion

Here, we describe the association results obtained for inflammasome SNPs in asthma cohort.

The distribution of genotypes was consistent with Hardy-Weinberg equilibrium for all SNPs. The linkage disequilibrium analysis determined that none of the studied SNPs is in strong LD in our cohort (D' < 0.95).

The association of inflammasome variants with asthma was first evaluated within the families (trios) by the Transmission Disequilibrium Test (TDT) (Table 2). The *NLRP1* variants rs11651270, rs12150220 and rs2670660 resulted associated to asthma, being the minor alleles rs11651270/C, rs12150220/T and rs2670660/G of the three SNPs significantly over-transmitted in studied families (p = 0.030, 0.019 and 0.046, respectively). When a combination of SNPs has been taken in account, we observe that the 3-SNPs combination including the major alleles rs11651270/T, rs12150220/A and rs2670660/A is less transmitted within the families (0.29 transmitted/0.69 untransmitted; p = 0.012). Of note this association resulted stronger when only the combination of rs11651270/T and rs12150220/A was considered (0.26 transmitted/0.67 untransmitted; p = 0.007).

**Table 1**
Demographic, clinical and serological data of studied cohort of asthma.

| Characteristic | Cases (n = 121) |
|---|---|
| Sex, male/female | 69/52 |
| Age (years), mean ± SD | 10.6 ± 3.5 |
| Asthma severity | |
| ●Mild intermittent, n | 15 |
| ●Mild persistent, n | 53 |
| ●Moderate persistent, n | 38 |
| ●Severe persistent, n | 15 |
| Pulmonary capacity (FEV1), mean ± SD | 0.87 ± 0.17 |
| Response to bronchodilators | |
| ●ΔFEV1, mean ± SD | 0.09 ± 0.09 |
| ●Good (ΔFEV1 ≥ 7%)/Bad (ΔFEV1 < 7%) | 49/54 |
| IgE total (AU/mL), n | 121 |
| ●positive/negative | 112/9 |
| ●mean ± SD | 987.44 ± 1038.79 |
| Dust and Mite Mix (Hx2) IgE (kAU/L), n | 72 |
| ●mean ± SD | 56.20 ± 40.76 |
| ●High/Low, n | 41/31 |
| Animal Dander Mix 2 (Ex2) IgE (kAU/L), n | 72 |
| ●mean ± SD | 0.89 ± 2.39 |
| ●High/Low, n | 13/59 |
| Mould Mix 2 (Mx2) IgE (kAU/L), n | 72 |
| ●mean ± SD | 0.31 ± 0.38 |
| ●High/Low, n | 33/39 |
| Cockroach (I6) IgE (kAU/L), n | 36 |
| ●mean ± SD | 1.36 ± 3.28 |
| ●High/Low, n | 6/30 |
| Cockroach American (I206) IgE (kAU/L), n | 36 |
| ●mean ± SD | 1.40 ± 3.49 |
| ●High/Low, n | 8/28 |
| Families (trios), n | 74 |
| Affected parents, n | 25 |
| ●Mother, n | 6 |
| ●Father, n | 19 |
| ●Mother and father, n | 1 |

Data are expressed as means ± standard deviation (SD), except where stated otherwise. FEV1: forced expiratory volume in one second; ΔFEV1: difference in FEV1 before and after bronchodilator treatment; AU: arbitrary units.

To determine the effect of inflammasome SNPs on severity and clinical parameters of asthma a multivariate analysis was performed in the cohort of 121 affected children considering separately all the variables adjusted for sex, age and race. *NLRP1* resulted associated to asthma severity (mild versus severe), being the minor alleles of rs11651270/C and rs2670660/G more frequent in severe than in mild asthma according with a dominant model of inheritance (p = 0.014, OR = 3.14; and p = 0.036, OR = 2.33, respectively). Confounding variables did not significantly affect the results ($p_{adj}$ = 0.013 and 0.029, respectively) (Table 3). The combination of the two minor alleles rs11651270/C and rs2670660/G resulted even more strongly associated with asthma severity (p = 0.008, OR = 2.29; $p_{adj}$ = 0.007, $OR_{adj}$ = 2.42) (Table 3).

Then we separately analysed total and specific IgE levels and pulmonary capacity of the patients. Once more, a *NLRP1* variant, rs2670660, resulted associated with a high IgE level (p = 0.026; $p_{adj}$ = 0.019) (Table 3). The combination of rs2670660_G/G and rs11651270_C/T genotypes resulted strongly associated to a high IgE level ($p_{adj}$ = 1.11 exp-4) (Table 3).

On the other hands, the 3′UTR *NLRP3* variant, previously associated to food-allergy and anaphylaxis (3), resulted associated to Hx2 IgE level in our cohort, according to an over-dominant model, being rs10754558_C/G significantly more frequent in patients with a low Hx2