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Polymorphisms in the *DAD1* and *OXA1L* genes are associated with asthma and atopy in a South American population

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ABSTRACT

Atopic asthma, which is characterized by the chronic inflammation and morbidity of airways, is a disease of great complexity, and multiple genetic and environmental factors are involved in its etiology. In the first genome-wide association study (GWAS) conducted in Brazil for asthma, a positive association was found between atopic asthma and a variant (rs1999071), which is located between the *DAD1* and *OXA1L* genes, although neither gene has previously been reported to be associated with asthma or allergies. The *DAD1* gene is involved in the regulation of programmed cell death, and *OXA1L* is involved in biogenesis and mitochondrial oxidative phosphorylation. This study aimed to evaluate how polymorphisms in *DAD1* and *OXA1L* are associated with asthma and markers of atopy in individuals from the Salvador cohort of the SCAALA (Social Change Asthma and Allergy in Latin America) program. The DNA of 1220 individuals was genotyped using the Illumina 2.5 Human Omni Bead chip. Logistic regression analyses were performed with PLINK 1.9 software to verify the association between *DAD1* and *OXA1L* polymorphisms and asthma and atopic markers, adjusted for sex, age, helminth infections and ancestry markers, using an additive model. The *DAD1* and *OXA1L* genes were associated with some of the evaluated phenotypes, such as asthma, skin prick test (SPT), specific IgE for aeroallergens, and Th1/Th2-type cytokine production. Using qPCR, as well as *in silico* gene expression analysis, we have demonstrated that some of the polymorphisms in both genes are able to affect their respective gene expression levels. In addition, *DAD1* was over-expressed in asthmatic patients when compared with controls. Thus, our findings demonstrate that variants in both the *DAD1* and *OXA1L* genes may affect atopy and asthma in a Latin American population with a high prevalence of asthma.

1. Introduction

Atopic conditions, such as atopic dermatitis, rhinitis and asthma, are some of the most common non-communicable diseases and are caused by chronic inflammatory reactions of type I hypersensitivity. They have a strong impact on quality of life and represent a substantial and growing socioeconomic burden for societies (Wight et al., 2017).

Asthma affects approximately 334 million people worldwide, and although it affects individuals across all age groups, it is one of the top 20 chronic conditions in the worldwide disability ranking among children 5–14 years of age (Asher and Pearce, 2014).

Asthma morbidity is characterized by variable and recurrent symptoms, including chronic inflammation, reversible obstruction of the airways and increased bronchial hyperresponsiveness. It is a complex disease with multiple genetic and environmental risk factors and a marked phenotypic heterogeneity (Yang et al., 2010).

In recent decades, large studies on asthma and atopy in diverse populations throughout the world have identified genetic polymorphisms that either serve as risk factors or offer protection for such conditions, especially in children (Strachan and Pearce, 2011). Although

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there exist genome-wide association (GWA) and candidate gene studies for asthma and atopy, the first GWA study for asthma in Brazil was only recently published, in 2015. In that study, the single-nucleotide polymorphism (SNP) rs1999071, located in an intergenic region between the *DAD1* and *OXA1L* genes in the 14q11 region, was described as a genetic risk factor for asthma (Costa et al., 2015).

The *DAD1* gene is known to be involved in the process of apoptosis, proving to be essential for the homeostasis and cell proliferation of various tissues (Nakashima et al., 1993). Apoptosis is known to be an essential process for normal development in multicellular organisms and to control inflammation, as well as immune homeostasis (Fuchs and Steller, 2015). A previous study has indicated that the increased expression of the *DAD1* gene in the thymus and the peripheral immune system is associated with an increase in the proliferation of peripheral T lymphocytes in mice (Hong et al., 1999).

OXA1L is a gene encoding the Oxa1 protein, which is involved in the biogenesis of mitochondrial oxidative phosphorylation machinery (Herrmann and Neupert, 2003; Hildenbeutel et al., 2008). Mitochondrial dysfunction may be involved in the physiopathology of many diseases, such as Kearns Sayre Syndrome, Leigh-syndrome or Leber's Hereditary Optic Neuropathy (Dankowski et al., 2016). It has been previously shown that mitochondrial dysfunction is commonly caused by polymorphisms in mitochondrial DNA and inadequate repair mechanisms (Wallace and Chalkia, 2013). In addition, (Mabalirajan and Ghosh, 2013) stated that there is a relationship between the immunopathology of asthma and mitochondrial biology, involving oxidative stress, calcium ion homeostasis and apoptosis pathways. It has been previously shown by (Jaffer et al., 2015) that mitochondria are relevant for remodeling in asthma, and dysfunctional mitochondria are evident in the smooth muscle cells of airways and in the lung epithelium of asthmatic patients (Leishangthem et al., 2013; Li and Shang, 2014).

Thus, in the present study, we have investigated the association between *DAD1* and *OXA1L* gene variants and asthma and allergic markers in an attempt to understand the mechanisms through which these variants affect asthma in Latin America to better characterize the disease and to suggest alternative pathways for the treatment of this illness.

2. Methods

2.1. Study population

The study was performed in children living in the city of Salvador, Northeast, Brazil, which has a population of approximately 2.8 million inhabitants. The study population consisted of approximately 1220 children, between 4 and 11 years of age, and has been described in previous studies of the group (Barreto et al., 2006; Figueiredo et al., 2009; Queiroz et al., 2017; Rodrigues et al., 2008). The children were recruited to the Salvador cohort of the SCAALA (Social Change Asthma and Allergy in Latin America) Program (Barreto et al., 2007).

To collect data, questionnaires based on the ISAAC (International Study of Asthma and Allergies in Childhood) phase 2 study (Asher et al., 1995) were used, with questions regarding asthma symptoms translated in to Portuguese (Alcantara-Neves et al., 2012), and the survey was conducted by properly trained field workers during home visits. Written consent was acquired from the parents or guardians of the children, and interviews were conducted in their presence. The project was approved by the ethics committees of the Federal University of Bahia (registry 003-05/CEP-ISC) and the National Council of Ethics in Research (CONEP, resolution number 15 895/2011).

2.2. Definition of asthma symptoms

Children were classified as asthmatic when parents or guardians reported wheezing associated with any of the following: the diagnosis of asthma by a physician at any time, wheezing with exercise during the previous 12 months, four or more episodes of wheezing during the previous 12 months, or night waking due to wheezing episodes during the previous 12 months. These classification parameters are more specific than the classification parameters most commonly reported by studies using the ISAAC questionnaire, which only consider reported wheezing during the previous 12 months. Children who did not meet these criteria were classified as non-asthmatic.

2.3. Specific IgE to aeroallergens

To determine the specific IgE levels, tests were performed using the ImmunoCAP assay (ThermoFisher, Waltham, Massachusetts, USA) for the following allergens: *Dermatophagoides pteronyssinus*, *Blomia tropicalis*, *Periplaneta americana* and *Blatella germanica*. Children who had specific IgE levels greater than or equal to 0.7 kU/L and/or a positive SPT for at least one test allergen were defined as atopic.

2.4. Blood collection and skin prick tests (SPT)

The children were evaluated by a medical team in a mobile clinic, where blood was collected, and skin prick testing was performed for the above mentioned aeroallergens, as well as for dog and cat epithelia and a fungal mix. Saline solution was used as the negative control, and a histamine solution at 10 mg/ml was used as the positive control. After 15 min, the reactions were read, and a diameter size of 3 mm greater than the negative control was considered positive.

Heparinized blood was collected, the plasma was prepared to measure specific IgE levels for the aeroallergens and whole blood cultures were analyzed to measure the production of cytokines in the cell supernatants.

2.5. Cell culture and measurement of IL-5, IL-10, IL-13 and IFN-γ by ELISA

Cells were cultured at a 1:4 dilution in RPMI medium (Gibco, Auckland, New Zealand) containing 10 mM of glutamine (Sigma-Aldrich, Inc., St. Louis, Missouri, USA) and 100 μg/ml gentamycin (Sigma-Aldrich, Inc. St. Louis, Missouri, USA) within 6 h following collection in heparinized tubes. For the detection of IL-5, IL-10, IL-13 and IFN-γ, cell cultures were maintained in a humidified atmosphere of 5% CO2 at 37 °C for 5 days, either without stimulation, to evaluate spontaneous production, or with stimulation using 2,5 μg/ml of *B. tropicalis* extract (Greer, Brazil). The concentrations of each cytokine were measured by sandwich ELISA, using commercial kits, following the manufacturer's recommendations (BD PharMingen, San Diego, CA, USA). There was no significant association between IL-5 and IL-10 cytokines (data not shown). The respective low and high detection limits (in pg/ml) were 15.6 and 500 for IFN-γ and 62.5 and 4000 for IL-13. Children were considered responsive when the measured values for both cytokines fell within the detection ranges.

2.6. Genotyping

The DNA was extracted from peripheral blood samples using a Flexigene@ DNA Kit (Qiagen, Hilden, Germany), and we performed genotyping using a commercial panel, a Illumina Bead Chip HumanOmni 2.5 Kit (www.illumina.com), through the Consortium EPIGEN-Brazil (https://epigen.grude.ufmg.br). The genetic information for *DAD1* was extracted from positions 23,033,807 to 23,058,143 (location: NC_000014.9) on chromosome 14. The genetic information for *OXA1L* was extracted from positions 23,235,731 to 23,240,998 (location: NC_000014.9) on chromosome 14. The following filters were applied for quality control: a genotyping call rate of less than 0.98; an imbalance of the Hardy–Weinberg equilibrium with a *P*-value of less than 10^{-4}; and a *P*-value for the minor allele frequency (MAF) of less than 1% (Laurie et al., 2010). A total of 33 markers were identified in