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ORIGINAL ARTICLE

Asthma and rhinitis have different genetic profiles for *IL13*, *IL17A* and *GSTP1* polymorphisms

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Abstract
Background: Asthma and rhinitis have a complex etiology, depending on multiple genetic and environmental risk factors. An increasing number of susceptibility genes are currently being identified, but the majority of reported associations have not been consistently replicated across populations of different genetic backgrounds.
Purpose: To evaluate whether polymorphisms of *IL4R* (rs1805015), *IL13* (rs20541), *IL17A* (rs2275913) and *GSTP1* (rs1695) genes are associated with rhinitis and/or asthma in adults of Portuguese ancestry.
Methods: 192 unrelated healthy individuals and 232 patients, 83 with rhinitis and 149 with asthma, were studied. All polymorphisms were detected by real time polymerase chain reaction (PCR) using TaqMan assays.
Results: Comparing to controls, significant association with asthma was observed for *GSTP1* rs1695 AA genotype (odds ratio (OR) – 1.96; 95% CI – 1.18 to 3.25; $p = 0.010$). The association sustains for allergic asthma (OR – 2.17; 95% CI – 1.23 to 3.80; $p = 0.007$). *IL13* rs20541 GG genotype was associated with less susceptibility to asthma (OR – 0.55, 95% CI – 0.33 to 0.94, $p = 0.028$). Among patients, *IL17A* rs2275913 AA genotype was less associated with asthma than with rhinitis (OR – 0.20; 95% CI of 0.07 to 0.56; $p = 0.002$). A similar association was found for *IL13* rs20541 GG genotype (OR – 0.48; 95% CI of 0.25 to 0.93; $p = 0.031$). There were no significant differences in the distribution of allelic and genotypic frequencies between patients and controls for the *IL4R* polymorphism' analyzed.

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Conclusion: These results support the existence of a significant association between *GSTP1* rs1695 and *IL13* rs20541 SNPs, with susceptibility to asthma, in the population studied. Different genotype profiles of *IL17A* and *IL13* genes seem to influence the clinical pattern of disease expression mainly confined to the upper airways, as rhinitis, or including the lower airways, as asthma.

Introduction

Asthma and asthma-related disorders, such as sinusitis and rhinitis, are complex diseases with strong genetic and environmental components.[1] Over the last decades their prevalence has been increasing worldwide, with a significant economic impact on health services.

Asthma is a common chronic inflammatory disease of the lower airways, characterized by reversible airflow obstruction, inflammation, persistent hyper-reactivity and airway remodeling.[2] Rhinitis is an upper airways inflammatory disease, often associated with asthma[3] and have been recognized as a risk factor for its development and severity.[4]

Genetic contribution to these phenotypes may account for 50–60%,[5,6] so dissecting genetic susceptible profiles may provide insight into the pathogenesis, allowing us to identify different sub-phenotypes and also contribute to finding new targeted therapies.

An increasing number of asthma susceptibility loci are continuously being identified, either by candidate gene studies or, more recently, by microarray-based whole genome approach, the genome-wide association (GWA) studies. Results are hampered by difficulties common to other complex diseases: inconsistent replication of results, functional association between identified loci and phenotype is not obvious, and most of all, allele penetrance is low and phenotype variance largely remains to be explained.[7–10] Most marker SNPs (single nucleotide polymorphisms) are localized in or near-by genes encoding proteins directly or indirectly involved in immunologic response. Recently, GWAs have revealed that different allergic diseases with common immunological physiopathology, also share susceptibility loci.[10]

Interleukin-4 (IL4) mediates important pro-inflammatory functions in allergic phenotypes and has principle responsibility for the IgE isotype switch by B lymphocytes.[11] IL4 is also involved in T helper type 2 (TH2) lymphocytes activation, induction of endothelium modifications, hypersecretion of mucus and with lung remodeling in chronic asthma.[11] IL4 action is mediated through activation of its receptor IL4R, a cell-surface heterodimeric complex.

Interleukin-13 (IL13) is one of the cytokines released by IL4 mediated Th2 induced cells and shares most of IL4 functions. IL13 production in the airway promotes the survival and migration of eosinophils, activation of macrophages, increased permeability and mucus production by airway epithelial cells and stimulates airways hyperresponsiveness.[12] Huang et al.[13] found that asthmatic and rhinitis patients submitted to allergen-challenge had a significant enhancement of *IL13* gene expression at mRNA and protein levels in bronchoalveolar lavage samples compared with the saline-challenged control sites.

Interleukin-17A (IL17A) is the best studied member of IL17 family of cytokines. It is mainly produced by activated T cells. Signaling through activation NF-kappaB and mitogen-activated protein kinases, IL17A regulates local tissue inflammation inducing the expression of pro-inflammatory cytokines, neutrophil-recruiting chemokines, cyclooxygenase-2 (COX2) and nitric oxide (NO). IL17A may also modulate the activation and proliferation of B cells, thus enhancing IgE production and has been associated with airway hyperresponsiveness and remodeling.[14,15]

SNPs from *IL4*, *IL4R* and *IL13* genes are among the polymorphisms most frequently implicated in susceptibility to asthma, rhinitis and allergic phenotypes in general,[16] with some of these SNPs being associated with increased serum IgE levels.[17,18] Up to now few studies are available on the association with genetic polymorphisms of IL17A family members.[19–21]

Reactive oxygen and nitrogen species, originated from air pollutants or released by inflammatory cells and stressed bronchial epithelia, are major contributors to asthma and asthma related phenotypes.[22] Glutathione-S-transferases (GSTs) are enzymes involved in the cellular detoxification of free radicals. A missense SNP in Glutathione S-transferase P1 (*GSTP1*), rs1695 (p.Ile105Val), has been associated with differences in enzyme activity and susceptibility to environmental-induced diseases, including asthma, but results remain controversial.[23,24]

Frequencies of DNA polymorphisms vary between populations of diverse ethnical origins, as their contributions to complex diseases, therefore, it is important to study different populations. In this study we evaluate the role of *IL4R* rs1805015, *IL13* rs20541, *IL17A* rs2275913 and *GSTP1* rs1695 in the susceptibility to asthma and rhinitis in an adult population of Portuguese ancestry.

Materials and methods

Clinical samples

This case–control study comprises 424 non related Caucasian Portuguese individuals, including 232 patients and 192 controls, randomly selected from a larger sample that answered a home inquiry about symptoms of asthma, rhinitis and sinusitis. Individuals willing to participate were than observed at the Immunoallergology Department of Coimbra University Hospitals.

The patient population was subdivided into two groups, according to the clinical diagnosis, using ARIA (Allergic Rhinitis and Its Impact On Asthma),[4] GINA (Global Initiative for Asthma)[2] and EP3OS (European Position Paper on Rhinosinusitis and Nasal Polyps 2007)[25] criteria: one group including patients with rhinitis but no asthma and another group including patients with persistent asthma, which could also have rhinitis. As explained in ''Results'' section, patients with both rhinitis and asthma were grouped with those who only had asthma. Controls had no symptoms related to respiratory system and no history of any allergic disease. Other known inflammatory or chronic conditions, including cancer, were exclusion criteria for both patients and controls. A skin prick test for 17 different inhalant allergens, selected according to the recommendations of the GA2LEN (Global Asthma and Allergy European Network),[26] was performed on all patients and those who had at least one positive result were considered allergic. The test was considered positive when wheal diameter was $\geq$3 mm and controls showed sufficient reaction.[26] Participants were considered allergic only in the presence of both symptoms and a positive skin prick test. The local ethics committee approved the study and patients provided informed consent.

Genotyping

DNA was extracted from 5 ml of frozen peripheral blood by standard methods. Characterization of the four SNPs was achieved by real time polymerase chain reaction (PCR) using the TaqMan assays (Applied Biosystems), iQTM Supermix (Bio-Rad Laboratories) and 10–30 ng of DNA, in a 7500 Fast Real-Time PCR System (Applied Biosystems). As positive controls, we used samples previously genotyped by automatic sequencing in an AbiPrism 3130 Genetic Analyser using BD v1.1 (Applied Biosystems) and Sequencing Analysis Software v5.2. The description of the four SNPs, the primers used in sequencing and TaqMan assay references are depicted in Table 1.

Statistical analysis

Statistical analysis was developed using the IBM® program SPSS® (Statistical Package for Social Sciences) version 20. Fisher exact-test, χ^2 was applied to analyze the difference between the three groups. Logistic regression models were used to control for potential confounders and to evaluate the associations between the risk of disease and genetic polymorphisms. All odds ratios (ORs) with 95% confidence intervals (CI) were adjusted for age, sex and smoking habits and $p < 0.05$ was considered statistically significant. When comparing rhinitis and asthma patients, ORs were also adjusted for atopy.

Results

A preliminary analysis revealed that for distribution of SNP variants, smoking habits and atopy (positive skin prick test results), there were no statistically significant differences between patients associating asthma and rhinitis and patients only with asthma (Supplementary Table 1). For this reason, and because asthma is considered a more aggressive phenotype, the two groups were joined together, so patients were further on classified as having rhinitis or asthma, as described in Table 2. The cohort of 83 patients with rhinitis had a mean age of 48.50 years (SD = 15.17) and 63.9% females. The group of 149 asthmatic patients was younger, with a mean age of 39.32 years (SD = 14.04) and 66.4% were females. The control group had a mean age of 56.06 years (SD = 19.48) and 52.1% females. According to results of skin prick test, allergy was more frequent among asthmatic patients (78.5%; Table 2).

The Hardy–Weinberg equilibrium was verified for the four polymorphisms ($p > 0.05$). The frequency of *GSTP1* rs1695 AA genotype was significantly higher in patients with asthma than in controls (49.0% vs. 33.9%; OR – 1.96, 95% CI – 1.18 to 3.25; $p = 0.010$) (Table 3). When only considering patients with allergic asthma (data not shown) results were similar (OR – 2.17, 95% CI – 1.23 to 3.80; $p = 0.007$). *IL13* rs20541 GG genotype was significantly less prevalent in asthma cases (62.6%) than in controls (70.8%) (OR – 0.55, 95% CI – 0.33 to 0.94; $p = 0.028$) (Table 3).

Among patients, *IL13* rs20541 GG genotype was less frequently associated with asthma than with rhinitis (OR – 0.48, 95% CI – 0.25 to 0.93; $p = 0.031$)(Table 4). The *IL17A* rs2275913 AA genotype was also significantly less frequent in asthmatic patients (5.4%) than in rhinitis patients (16.9%)(OR – 0.20, 95% CI – 0.07 to 0.56; $p = 0.002$) (Table 4).

For *IL4R* rs1805015, there were no significant differences in the distribution of allelic and genotypic frequencies

Table 1 SNPs evaluated.

Gene	SNP	Primer sequence[a]	TaqMan assay ID
IL4R	rs1805015; TCC>CCC missense (p.Ser503Pro)	F 5′ ATGCCTTCTTCCACCTTC R 5′ CATCTCGGGTTCTACTTCC	C__8903092_20
IL13	rs20541; CAG>CGG missense (p.Gln144Arg)	F 5′ CCGTGAGGACTGAATGAG R 5′ CACAGGCTGAGGTCTAAG	C__2259921_20
IL17A	rs2275913; −197G>A[b] (regulatory region)	F 5′ ATGACACCAGAAGACCTAC R 5′ TGTGCCTGCTATGAGATG	C__15879983_10
GSTP1	rs1695; ATC>GTC missense (p.Ile105Val)	F 5′ TGTGGCAGTCTCTCATCC R 5′ GCAGGTTGTGTCTTGTCC	C__3237198_20

[a] Primers used in genotyping by sequencing.
[b] NM_002190.2:c−197G>A.

Table 2 Characteristics of patients and controls.

	Rhinitis $n = 83$ (%)	Asthma $n = 149$ (%)	Controls $n = 192$ (%)	p-Value
Gender				
Male	30 (36.1)	50 (33.6)	92 (47.9)	0.005
Female	53 (63.9)	99 (66.4)	100 (52.1)	
Age (years)				
Mean (SD)	48.50 (15.17)	39.32 (14.04)	56.06 (19.48)	<0.001
Smoking habits				
No smoking	54 (65.9)	104 (71.2)	121 (64.4)	NS
Smoking	28 (34.1)	42 (28.8)	67 (35.6)	
Skin test positive[a]	40 (48.2)	117 (78.5)	NA	<0.001

[a] Test with at least one positive result.
NA – not available.
SD – standard deviation.
NS – without statistical significance.

between patients and controls (Table 3) or between the two disease phenotypes (data not shown).

Discussion

In this work we evaluate the role of polymorphisms of four genes, *IL4R*, *IL13*, *IL17A*, and *GSTP1* in the susceptibility to two related phenotypes, asthma and rhinitis.

As expected,[27] patients were mostly of female gender. In agreement with previous studies,[28] our results suggest that most patients with asthma have allergen sensitization.

Comparing patients and controls, *GSTP1* rs1695 AA genotype conferred about twofold risk for developing asthma and allergic asthma. These results of a Portuguese population are in accordance with previous studies that have associated the A variant with a higher risk,[29] or the G (Val) variant with a lower risk[30,31] to develop asthma and allergic phenotypes. Yet a meta-analysis[23] only suggested a possible, weak protective effect of the G (Val) allele. Like other authors, we found no evidence of a correlation between *GSTP1* SNP and rhinitis.[32]

GSTs play important roles in airway antioxidant defenses[33] and *GSTP1* enzyme contributes to more than 90% of GST-derived enzyme activity in human lung epithelium.[34] The rs1695 SNP has been associated with differences in GSTP1 substrate affinities.[35] A recent study[36] showed that asthma patients who have homozygosity for A (Ile) allele

Table 3 Comparative study of genotypic frequencies in controls and patients.

	Controls n (%)	Rhinitis n (%)	p-Value[a]	Asthma n (%)	p-Value[b]	AdjOR[c,b] (95% CI)
GSTP1 rs1695						
Ile/Ile (AA)	65 (33.9)	35 (42.2)	0.260	73 (49.0)	0.010	1.96 (1.18–3.25)
Ile/Val (AG)	97 (50.5)	40 (48.2)	0.849	61 (40.9)	0.418	
Val/Val (GG)	30 (15.6)	8 (9.6)	0.202	15 (10.1)	0.418	
IL13 rs20541						
Arg/Arg (GG)	136 (70.8)	60 (73.2)	0.686	92 (62.6)	0.028	0.55 (0.33–0.94)
Gln/Arg (AG)	52 (27.1)	20 (24.4)	0.694	50 (34.0)	0.908	
Gln/Gln (AA)	4 (2.1)	2 (2.4)	0.948	5 (3.4)	0.908	
IL17 rs2275913						
GG	94 (49.0)	28 (33.7)	0.068	69 (46.6)	0.552	
AG	81 (42.2)	41 (49.4)	0.532	71 (48.0)	0.978	
AA	17 (8.9)	14 (16.9)	0.072	8 (5.4)	0.230	
IL4R rs1805015						
Ser/Ser (TT)	140 (73.3)	65 (78.3)	0.374	115 (77.2)	0.939	
Ser/Pro (TC)	43 (22.5)	15 (18.1)	0.447	30 (20.1)	0.614	
Pro/Pro (CC)	8 (4.2)	3 (3.6)	0.707	4 (2.7)	0.199	

[a] Controls vs rhinitis
[b] Controls vs asthma
[c] AdjOR – Adjusted Odds Ratio for age, gender and smoking habits.

Table 4 Comparative study of *IL17A* and *IL13* polymorphisms genotypic frequencies between the two patient groups.

	Rhinitis *n* (%)	Asthma *n* (%)	*p*-Value	AdjOR (95% CI)[a]
IL13 rs20541				
Arg/Arg (GG)	60 (73.2)	92 (62.6)	0.031	0.48 (0.25–0.93)
Gln/Arg (AG)	20 (24.4)	50 (34.0)	0.797	
Gln/Gln (AA)	2 (2.4)	5 (3.4)	0.797	
IL17 rs2275913				
GG	28 (33.7)	69 (46.6)	0.97	
AG	41 (49.4)	71 (48.0)	0.97	
AA	14 (16.9)	8 (5.4)	0.002	0.20 (0.07–0.56)

[a] AdjOR – Adjusted Odds Ratio for age, gender, atopy and smoking habits.
For the *IL4R* and *GSTP1* polymorphism' analyzed, there were no significant differences in the distribution of genotypic frequencies between patients.

are more likely to be affected by air pollutants. These findings substantiate the functional relevance of the SNP,[37] and support our results. Yet, we are aware that this genetic effect can be affected by environment-induced epigenetic modifications that influence gene expression and ultimately, the level of enzymatic activity.[38,39] Epigenetic regulation is tissue-specific and may account for some of the differences in GSTP1 role between upper and lower airways.

For *IL13* SNP, our results suggest that homozygosity for G allele, corresponding to arginine (Arg) variant, may decrease susceptibility to asthma. *IL13* polymorphisms have repeatedly being associated with increased IgE serum levels, allergy and asthma.[16,40] The association of rs20541 with allergic rhinitis was reported in a recent meta-analysis of an Asian population.[41]

IL13 rs20541 (CAG>CGG) results in the non-conservative replacement of a neutral glutamine (Gln) for a positively charged arginine (Arg) in the IL13 α helix of D domain, the region that is thought to interact with IL4Rα/IL13Rα1 heterodimers.[42] The A allele has been associated with increased IL13 expression[43] and a lower affinity for the IL13 receptor α2 chain (IL13Rα2), which is a decoy receptor that antagonizes inflammation and tissue remodeling.[44] These observations point to a lower inflammatory activity of arginine variant (G allele) comparing with glutamine (A allele), supporting the association with a decreased susceptibility to asthma.

In contrast to other studiess,[17,41] we could not confirm the same correlations for rhinitis. The difference in results may be explained by the fact that upper airways patency is largely influenced by vascular tone, whereas in the lower airways, smooth muscle function is determinant.[45] IL13 was shown to directly interfere with airway smooth muscle cell responsiveness by enhancing agonist-induced contractility and calcium signals, a mechanism that may be mediated by stromal interaction molecule-1 (STIM1), a sarcoplasmic reticulum protein involved in the regulation of intracellular Ca^{2+} concentrations.[46]

Comparing both groups of patients, *IL17A* rs2275913 AA genotype was less frequently associated with asthma than with rhinitis, reinforcing the difference between the genetic profiles of these phenotypes. This SNP is located in 5′ regulatory region, within a binding motif for the nuclear factor of activated T cells (NFAT), which is a critical regulator of the *IL17A* promoter. Peripheral blood level determination and gene reporter assays showed that the A variant was associated with increased levels of IL17A expression and displayed a higher affinity for NFAT.[47]

For rhinitis, our data concur with the functional relevance of A variant. IL17A is known to be a proinflammatory, leukocyte-derived cytokine that targets epithelial cells[48] and that seems to play a central role in rhinitis.[14] IL17A expression in the nasal mucosa was associated with the pathophysiology of allergic rhinitis, including disease severity and local eosinophilia.[49]

In asthma, increased expression of IL17A has been described mainly in severe and non-allergic forms.[50,51] The majority of patients included in our study had moderate asthma, which may account for the negative association observed with the higher inflammatory activity A allele. Reinforcing our results, Lei et al.[52] describe similar serum levels of IL-17A between asthmatic and normal controls. Globally, controversial results have been published, with studies suggesting that different polymorphisms of *IL17A* and *IL17F* may contribute both to susceptibility or resistance to asthma or allergic rhinitis.[19–21]

No significant association was observed between *IL4R* rs1805015 polymorphism with rhinitis or asthma. Although this association was confirmed in recent GWA studies,[9] contradictory reports exist and results frequently differ between populations.[16,18]

The difference in results between our data and some previous reports is a common issue in complex disease research. Differences in population genetic backgrounds, in sample size and selection, phenotype classification, in environmental exposure and epigenetic mechanisms, contribute to the results divergence.

To overcome limitations of this study, in future work it will be important to enlarge the population samples to be analyzed and to define more specific and homogeneous phenotypes.

Conclusions

We describe the existence of a significant association between *GSTP1 rs1695* and *IL13* rs20541 polymorphisms, respectively with higher and reduced susceptibility to

asthma. For patients that develop reactive inflammatory respiratory diseases, *IL17A* rs2275913 and *IL13* rs20541 polymorphisms influence the predominant upper airways (rhinitis) or lower airways (asthma) clinical pattern. These results also support the hypothesis that the genetic susceptibility profiles of asthma and isolate chronic rhinitis are not equivalent.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors have obtained the written informed consent of the patients or subjects mentioned in the article. The corresponding author is in possession of this document.

Conflicts of interest

None of the authors have any conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary material associated with this article can be found in the online version available at doi:10.1016/j.rppnen.2016.06.009.

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