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Relationship between the 17q21 locus and adult asthma in a Czech population

Barbara Hrdlickova [a,b], Lydie Izakovicova Holla [a,*]

[a] Department of Pathophysiology, Faculty of Medicine, Masaryk University, Brno, Czech Republic
[b] Department of Surgery, University Hospital Brno-Bohunice, Brno, Czech Republic

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ABSTRACT

Several whole-genome association studies have shown a significant link between childhood asthma and the 17q12 chromosome region. We selected tagging single nucleotide polymorphisms (SNPs) in the ORMDL3 gene (17q12) to investigate gene variability in relation to adult allergic asthma and asthma/atopy traits in a Czech Caucasian population of adults. We conducted a case-control association study comprising 668 unrelated subjects (337 asthmatic and 331 control subjects). Four selected SNPs (rs17608925, rs12603332, rs8076131, and rs3169572) were genotyped using the TaqMan SNP Genotyping Assays. The single locus analysis showed only a borderline association between rs3169572 variant and asthma ($p = 0.030$, $p_{corr} > 0.05$). However, seven different haplotypes were identified; among them, the TTAA haplotype was marginally associated with asthma ($p = 0.045$, $p_{corr} > 0.05$) and TCAG haplotype was significantly associated with asthma in males ($p = 0.009$, $p_{corr} < 0.05$, odds ratio = 1.48, 95% confidence interval = 1.10–2.00). In addition, associations between the ORMDL3 genotypes and the total IgE level ($p = 0.05$, $p_{corr} > 0.05$) and hypersensitivity to the pollen ($p = 0.007$, $p_{corr} < 0.05$) were established. However, no relationship between ORMDL3 SNPs and the pulmonary functions was found ($p > 0.05$). These findings suggest that the genetic variability in the 17q21 region may be one of the risk factors also for adult asthma, especially in male individuals.

1. Introduction

Asthma is a chronic inflammatory disease of the airways, characterized by airway hyperresponsiveness and widespread but variable airway obstruction that is often reversible. The disease results from complex interactions between genetic and environmental factors. Recent genome-wide association studies have shown linkage of childhood asthma to the genomic region 17q12–17q21 [1–3] where the ORMDL3 gene is located.

The ORM1-like protein 3 gene (ORMDL3) encodes a protein containing four transmembrane domains located in the endoplasmic reticulum (ER) membrane. Although present knowledge of the ORMDL3 function is limited, a study in yeast has shown that the gene product may be involved in protein folding [4]. New findings suggest that ORMDL3 is involved in endoplasmatic reticulum-mediated Ca^{2+} signaling and facilitation of ER-mediated inflammatory responses [5], this can explain the reported association of ORMDL3 with asthma and other inflammatory disorders such as Crohn's disease [6]. Very recently, Breslow et al. described ORM proteins as negative regulators of sphingolipid synthesis; they form a conserved complex with serine palmitoyltransferase, the rate-limiting enzyme in sphingolipid production [7]. However, how this relates to asthma still remains unknown. Interestingly, the greater risk of asthma conferred by ORMDL3 variants has been associated with tobacco smoking [3], an environmental disease modifier inducing unfolded-protein response [8].

The original investigation describing the ORMDL3 gene as a risk factor for childhood asthma has been followed by several replication studies and several polymorphisms at the 17q21 locus have been associated with childhood asthma and its phenotypes in different populations over the years [1–3, 9–15] (Table S1). The aim of this investigation was to elucidate the role of ORMDL3 gene polymorphisms in allergic asthma and asthma/atopy traits in a Czech population of adults.

2. Subjects and methods

2.1. Subjects

This case-control study comprised 668 unrelated Czech Caucasian subjects (Table 1). All subjects filled in a modified American Thoracic Society respiratory questionnaire [16] considering lifetime symptoms suggestive of asthma, rhinoconjunctivitis, and atopic eczema/dermatitis syndrome, extended with additional questions regarding symptoms and therapy and other diseases. A total of 337 patients with clinically manifested allergic bronchial asthma met, at minimum, one of the following criteria: (1) a physician's diagnosis of asthma (with no conflicting diagnosis); (2) the presence of at least two self-reported symptoms (cough, wheeze, shortness of breath); (3) current use of asthma medications; or (4)

* Corresponding author.
E-mail address: holla@med.muni.cz (L.I. Holla).

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Table 1
Clinical characteristics of study subjects

Characteristic[a]	Asthmatic subjects (n = 337)	Control subjects (n = 331)
Age (y), mean ± SD)	30.7 ± 14.8	38.1 ± 16.2
Gender: male/female	178/159	170/161
Asthma only (%)	54 (16.0)	0
Asthma and rhinitis (%)	162 (48.1)	0
Asthma and dermatitis (%)	22 (6.5)	0
Asthma, rhinitis, and dermatitis (%)	99 (29.4)	0
Asthma, intermittent (%)	28.5	0
Asthma, persistent (%)	71.5	0
FVC (% predicted)[b]	93.1 ± 13.8	–
FEV_1 (% predicted)[b]	92.6 ± 14.4	–
FEV_1/FVC (%)[b]	84.1 ± 25.2	–
Smoking, no/yes (%)	86.1/13.9	71.6/28.4[c]
Serum log IgE	2.15 ± 0.64	1.54 ± 0.56[c]
Food allergy, no/yes (%)	83.6/16.4	100.0/0[c]

[a]Data are expressed as means ± SD unless stated otherwise.
[b]Spirometry was performed in asthmatic subjects only.
[c]$p < 0.001$ (controls vs asthmatic groups).

either bronchial hyperresponsiveness, defined as a 20% decrease in forced expiratory volume in 1 second (FEV_1) after inhalation of 25 mg/ml of methacholine or reversibility to inhaled bronchodilator, defined as a 12% increase in baseline FEV_1 after inhalation of a bronchodilator (salbutamol) according to the international standards [17,18]. The diagnosis and classification of the clinical severity of asthma (intermittent, mild, moderate, and severe persistent categories) was established according to the Global Initiative for Asthma (GINA) guidelines [19]. The diagnosis of concomitant allergic rhinitis was based on coordination between a typical history of allergic symptoms of either perennial (non-seasonal) or seasonal (hay fever) rhinitis and positivity of the diagnostic test for IgE-mediated allergy [20]. Atopic eczema/dermatitis syndrome (AEDS) was defined according to the diagnostic criteria for atopic eczema initially proposed by Hanifin and Rajka [21]. Atopy was defined as the presence of positive skin test reaction (≥3 mm greater than reaction to saline) to one or more of the common allergens (house dust mite, common mixed grass and tree pollens, mixed molds, cat and dog dander, together with histamine and normal saline as positive and negative controls, respectively) and/or increased specific serum IgE levels (>0.35 kU/l by AlaSTAT test (DPC Biermann, Bad Nauheim, Germany) produced in response to one or more of the common allergens, including *Dermatophagoides farinae*, grass pollens, animal dander, and molds) and/or total serum IgE levels above normal values (>150 IU/ml in nonsmoking adults measured by the nephelometric test (Dade-Behring, Mannheim, Germany) as described previously [22]. A total of 15 different antigens were tested in variable subgroups of patients exhibiting symptoms suggestive of atopy. Asthmatic patients also underwent spirometric measurement of their forced vital capacity (FVC) and FEV_1.

The control group consisted of 331 healthy subjects who met the following criteria: (1) no prior or currently established diagnosis of asthma and/or rhinitis and/or dermatitis, (2) no history of wheezing, shortness of breath and other symptoms of allergic diseases, such as nasal and skin symptoms, (3) no use of anti-asthma medications, and (4) absence of first-degree relatives with a history of asthma or atopy. Allergen skin prick testing was not performed in the control subjects; therefore, these data were not included in the study.

This study was approved by the Committee for Ethics at the Faculty of Medicine, Masaryk University, and informed consent was obtained from all participants, in line with the Declaration of Helsinki, before inclusion in the study.

2.2. Genotyping

Genomic deoxyribonucleic acid (DNA) was extracted from peripheral blood leukocytes by the phenol-chloroform method using the proteinase K. Tagging single nucleotide polymorphisms (SNPs) were selected from HapMap (http://www.hapmap.org/, HapMap Data Rel24/phaseII Nov08, on NCBI B36 assembly, dbSNP b126, Population: CEU, Pairwise methods: Tagger Pairwise, MAF = 0.05, $r^2 = 0.8$) using the implemented algorithm. Four SNPs (rs17608925, rs12603332, rs8076131, and rs3169572) were included in this study and were genotyped using the TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, CA).

2.3. Statistical analysis

The power of study to detect significant differences between study groups was calculated according to Whitley and Ball [23] and Altman [24]. Power calculation was done prospectively on the bases of HapMap data and for Fisher's exact test as a principal method used for comparisons of our allele frequencies. Based on HapMap data (Table S2), the minor allele frequencies of the markers used ranged from 0.07 to 0.48. Power estimates were based on 10% differences in allele frequencies in objective, significant outcomes of comparisons; the test power finally ranged from 0.35 to 0.80.

Differences in allele frequencies were tested by Fisher exact test, and genotype distributions and Hardy–Weinberg equilibrium (HWE) by χ^2 test (Table 2). The Kruskal–Wallis analysis of variance (ANOVA) was used for the evaluation of total/specific IgE levels. Where appropriate, the Bonferroni correction was used to adjust the level according to the number of independent comparisons to the overall value of 0.05. Adjusted p values for particular analyses are denoted as p_{corr}.

PHASE 2.1 was used for *in silico* estimation of haplotype frequencies, and differences in haplotype distributions were assessed by a permutation test. To examine the linkage disequilibrium (LD) between all SNPs, pairwise LD coefficients D′ and r^2 were calculated (Table 3) using the SNP Analyzer 2 program (available at http://snp.istech.info/istech/board/login_form.jsp).

Contingency tables, odds ratios (OR), 95% confidence intervals (CI), and significance values were estimated using STATISTICA 9.0 (Statsoft, Inc., Tulsa, OK).

3. Results

A total of 337 patients with clinically manifest asthma and 331 healthy control subjects were enrolled in this study (Table 1). Smoking was significantly less common in asthmatic subjects (13.9%) in comparison with controls (28.4%, $p < 0.001$). As expected, total IgE levels were higher in asthmatic subjects (log IgE = 2.15 ± 0.64) than in healthy controls (log IgE = 1.54 ± 0.56) ($p < 0.001$).

The comparison of the observed and expected genotype frequencies of each of the tested polymorphisms revealed that all groups were in Hardy–Weinberg equilibrium with nonsignificant χ^2 values except for rs17608925 in asthmatic subjects. In the single locus analysis, no significant differences were found in allelic or genotype distributions of the tagged SNPs ($p > 0.05$) in this case-control study, with the exception of rs3169572 variant, where a marginal difference in allelic frequency was determined (Table 2). The frequency of A allele was lower in asthmatic subjects compared with controls (4.3% vs 6.8%, $p = 0.030$, $p_{corr} > 0.05$). Subanalysis performed separately in groups of male and female subjects showed a significant difference in the allele frequency of the rs12603332 variant in male subjects; the C allele was more frequent in asthmatic subjects (54.2% vs 46.2%, $p = 0.020$, $p_{corr} > 0.05$) and the G allele of the rs8076131 polymorphism in controls (48.8%