17q21 variant increases the risk of exacerbations in asthmatic children despite inhaled corticosteroids use

Short title: 17q21 and risk of asthma exacerbations in children

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Letter to the editor,

Approximately 25% of the asthmatic children suffer from uncontrolled asthma despite regular use of inhaled corticosteroids (ICS) [1]. Variation within the 17q21 locus is the strongest genetic determinant for childhood-onset asthma [2]. Recently, the influence of this locus on treatment outcomes has been shown in several studies [3–5]. The Pharmacogenomics in Childhood Asthma (PiCA) consortium is a multi-ethnic consortium that brings together data from ≥14,000 asthmatic children/young adults from 12 different countries to study the pharmacogenomics of uncontrolled asthma despite treatment [6]. In 14 PiCA populations (with over 4000 asthmatic patients), we studied the association between variation, in the 17q21 locus, and asthma exacerbations despite ICS use. We specifically focused on rs7216389, a single nucleotide polymorphism (SNP) in the 17q21 locus strongly associated with childhood asthma and initially identified by Moffatt et al. [2].

Ten PiCA studies included patients with non-Hispanic European origins, two included Hispanic patients, one African-American and one included east-Asian patients. Additional details of the study populations can be found in the online supporting information. Two outcomes were assessed: 1) asthma-related hospitalizations/emergency department visit (ED) visits and 2) short courses of oral corticosteroid (OCS) use reported by the parent/child at the study visit or based on completed study questionnaires. Age, gender, genotype data and exacerbation data were available for 4,529 steroid-treated children and young adults (Table 1). Logistic regression analysis was used to assess the risk of exacerbations when carrying rs7216389. Due to potential heterogeneity between cohorts, the odds ratios (ORs) were meta-analyzed with the inverse variance weighting method assuming random-effects. See online supporting information for more detail.

The risk allele (T) frequency was highest in East-Asians (n= 182, T = 0.81), followed with African-Americans (T=0.79, n=468) and Hispanics (T=0.66, total n=916) and it was less frequent in patients with European ancestry (ranged between 0.54-0.62, total n = 2,963). The genotype distribution of the

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SNP was in Hardy-Weinberg equilibrium in all cohorts. There was a low to moderate heterogeneity between studies (Fig 1). Exacerbation rates ranged between 6.5% (PACMAN) and 77.2% (HPR) for OCS use and 6% (PACMAN) and 58% (GALA II and HPR) for hospitalizations/ED visits.

Thirty percent (1,378 out of 4,454) of the patients reported hospitalizations/ED visits. In the meta-analysis of 13 studies, rs7216389 was statistically significantly associated with asthma-related ED visits/hospitalizations, (summary OR per increase in risk allele:1.32, 95%CI:1.17-1.49, p<0.0001, $I^2=3.9\%$) (Fig 1). In a subgroup analysis, the effect estimates for hospitalizations/ED visits were approximately the same for both non-Hispanic whites (n= 2,888, OR:1.33, 95%CI:1.10-1.61, p=0.004, $I^2=30.2\%$) and Hispanics (n= 916, OR: 1.31, 95%CI:1.06-1.63, p=0.01, $I^2=0.00\%$). Thirty-one percent (1,269 out of 4,050) of the patients reported OCS use/high dose ICS. In the meta-analysis of the nine studies, the rs7216389-T was statistically significantly associated with an increased risk of OCS use/ high dose ICS (summary OR per increase in variant allele:1.19, 95%CI: 1.04-1.36, p=0.01, $I^2=22.8\%$) (Fig 1). Rs7216389 was associated with OCS use in the meta-analysis of seven European studies (n=2,492, OR:1.26, 95%CI:1.09-1.45, p=0.002, $I^2=6.2\%$) but not in Hispanics (n= 916, OR: 0.96, 95%CI:0.76-1.22, p=0.7, $I^2=0.00\%$). Differences in the minor allele frequencies and LD structures among different ethnicities can influence results of the association studies [7]. This could be one of the potential explanations why we did not find a significant association in African-Americans and patients from Singapore.

A sensitivity analysis was performed to investigate this association in children ≥5 years of age. When excluding children <5 years of age in the meta-analysis, the results remained significant. In the meta-analysis of 13 studies, the SNP was associated with asthma-related hospitalization/ED visits (n= 4,254, OR: 1.32, 95%CI: 1.18-1.49, p<0.0001, $I^2=0.0\%$) (figure S1). Regarding OCS use, 10 studies collected data on patients ≥5 years of age (n= 3,771). In the meta-analysis of 10 studies, rs7216389...
was associated with the OCS use (summary OR: 1.20, 95%CI: 1.05-1.38, p=0.01, I² = 21.7%) (figure S2).

We also performed a meta-analysis of the studies that had sufficient data available on preschool children (2-4 years of age). Although the effect estimates in younger children were in the same direction for both outcomes, the results were not statistically significant (figure S3 & S4). All preschool studies solely included European children.

Altered expression of ORMDL3 and GSDMB by 17q21 locus variants may play a key role in childhood asthma onset [2, 8]. Two 17q21 asthma-risk variants (rs4065275 and rs12936231) in high linkage disequilibrium (LD) with rs7216389 were reported to switch CTCF-binding sites that resulted in increased expression of ORMDL3 in CD4+ T cells which subsequently reduced interleukin-2 production [9].

Rs7216389 have previously shown to be associated with exacerbations [3] and poor lung function in Caucasian children using ICS [4]. Even though in our study Caucasians were the largest group, this study is the largest multi-ethnic population evaluating the association between 17q21 variant and asthma exacerbations in ICS users. Rs7216389 seems to increase bronchial responsiveness and therefore exacerbation rates in children [10], suggesting that carriers of rs7216389 might have a more severe form of asthma. However, by adding BTS treatment steps as a marker of disease severity to the model, we argue that the association reflects, at least partly, poor response to ICS.

Limitations of the study include the use of retrospective reporting of exacerbations in the observational cohort studies. However, the effect was also observed in a clinical trial population (CAMP), where exacerbations were reported prospectively. Hence, we do not believe that using
retrospective data has significantly influenced the results. Since not all studies had data available on both hospitalizations/ED visits and OCS use, we did not combine the two outcomes in our analysis. Furthermore, since information on treatment adherence was not available in all included studies, it was not considered in the analysis.

We show that 17q21, a widely replicated asthma susceptibility locus, is also associated with an increased risk of exacerbations in children/young adults treated with ICS. A better understanding of the molecular mechanisms underlying exacerbation-prone phenotype of pediatric asthma could lead to a better classification of different pediatric asthma phenotypes and the identification of novel treatment targets.

References


5. Farzan N, Vijverberg SJH, Arets HG, Raaijmakers JAM, Maitland-van der Zee AH.

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Authors’ contribution

AHM designed and managed the PACMAN study, contributed to the design of the PiCA consortium, supervised data analysis, reviewed and corrected the manuscript.

SJV designed and managed the PACMAN study, contributed to the design of the PiCA consortium, supervised data analysis and wrote the manuscript in collaboration with NF.

NF performed the meta-analyses and analyzed the PACMAN data and wrote the manuscript in collaboration with SJV.

EB contributed to results interpretation and reviewed and corrected the manuscript.

EM contributed with BAMSE data and samples, reviewed and corrected the manuscript.

CS performed the genetic analyses in BAMSE, reviewed and corrected the manuscript.

SKM analyzed the BAMSE data, reviewed and corrected the manuscript.

RT performed genotyping for BREATHE and PAGES.

CNP coordinated genotyping BREATHE and PAGES and contributed to results interpretation. Reviewed and corrected the manuscript.

PS contributed to results interpretation, reviewed and corrected the manuscript.

SM contributed to design and management of the BREATHE study. Reviewed and corrected the manuscript.

KB contributed to the study design, patient recruitment, and examination, data interpretation of the COPSAC2000 study. KB also reviewed and corrected the manuscript.

HB involved in study design and data interpretation of the COPSAC2000 study.

AS performed the data analyses of COPSAC2000, reviewed and corrected the manuscript.

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NHP and MP-Y analyzed the GALA II and SAGE II data, reviewed and corrected the manuscript.

EGB contributed to the design and recruitment of patients and provided reagents and materials of the GALA II and SAGE II studies.

SMT contributed to CAMP data analyses, read and approved the manuscript.

KGT contributed to design and management of the CAMP study, reviewed and corrected the manuscript.

KMV contributed to design and management of the ESTATe study, reviewed and corrected the manuscript.

LK contributed to ESTATe data analyses, read and corrected the manuscript.

MK contributed to design and management of followMAGICS, reviewed and corrected the manuscript.

MS contributed to followMAGICS data analyses, read and approved the manuscript.

GC contributed to recruitment, data collection and results interpretation of the HPR, reviewed and corrected the manuscript.

JCC designed the HPR study, contributed to data analysis, results interpretation and review of the manuscript.

MMC contributed to recruitment and data collection of the HPR study, results interpretation and review of the manuscript.

EF contributed to data cleaning and analysis of the HPR study, results interpretation and review of the manuscript.

ST is the data custodian for the PAGES cohort, provided data from the cohort and reviewed and corrected the manuscript.
BF contributed to data and pharmacogenomic analysis, results interpretation of the PASS study reviewed and corrected the manuscript.

DH contributed to design and management of the PASS study, performed data and pharmacogenomic analysis, reviewed and corrected the manuscript.

RS conceived PASS, study management, and oversight and performed data analysis.

MP conceived PASS, study management, and oversight and performed data and pharmacogenomic analysis

FTC designed and maintained the Singapore cohort and dataset; designed and coordinated all experimental work relating to the study; and analyzed the data and reviewed the manuscript.

WCC coordinated the clinical assessment of the Singapore cohort and follow up of the clinical cohort.

YYC performed the genotyping and analysis of the Singapore cohort.

VB involved in patients’ enrollment, clinical data collection, and analysis of the Slovenian cohort.

UP contributed to clinical data analysis, genotyping experiment design, genotype data analysis of the Slovenian cohort.

KR contributed to clinical data analysis, Genotyping, genotype data analysis of the Slovenian cohort.

All authors read and approved the final manuscript.
Conflict of interest:

AHM reports an unrestricted research grant from GSK, during the conduct of the study; she was a member of an advisory board for AstraZeneca, outside the submitted work. MPY reports grants from Spanish Ministry of Economy and Competitiveness (RYC-2015-17205), from Instituto de Salud Carlos III (ISCIII, AC15/00015), and from ERACoSysMed 1st Joint Transnational Call (SysPharmPedia), during the conduct of the study. NHP reports grants from Instituto de Salud Carlos III (ISCIII) and co-funded by the European Social Funds from the European Union (ESF) “ESF invests in your future”, during the conduct of the study. KGT reports grants from U.S. National Institutes of Health, during the conduct of the study. SJV reports grants from Stichting Astmabestrijding, during the conduct of the study; and PACMAN cohort was funded by a strategic alliance between Utrecht Institute for Pharmaceutical Sciences and GSK. The other authors have no other conflict of interests that are directly relevant to the content of this manuscript.
Table 1. Characteristics of the study populations

<table>
<thead>
<tr>
<th>Patient Characteristic</th>
<th>BAMSE# (n=122)</th>
<th>BREATHE (n=808)</th>
<th>CAMP# (n=172)</th>
<th>COPSAC2000*# (n=54)</th>
<th>eSTATE (n=95)</th>
<th>followMAGICS# (n=150)</th>
<th>GALA II# (n=744)</th>
<th>HPR# (n=172)</th>
<th>PACMAN (n=665)</th>
<th>PAGES (n=308)</th>
<th>PASS# (n=390)</th>
<th>SAGE II# (n=468)</th>
<th>SCGIES# (182)</th>
<th>Slovenia# (n=199)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs), mean (SD)</td>
<td>8.37 (0.41)</td>
<td>9.8 (4.0)</td>
<td>8.8 (2.1)</td>
<td>3.3 (1.0)</td>
<td>10.8 (4.3)</td>
<td>17.13 (3.03)</td>
<td>12.1 (3.2)</td>
<td>9.8 (2.7)</td>
<td>8.7 (2.3)</td>
<td>9.2 (3.8)</td>
<td>11.1 (4.0)</td>
<td>13.5 (3.4)</td>
<td>13.35 (5.09)</td>
<td>10.9 (3.41)</td>
</tr>
<tr>
<td>Male gender, % (n)</td>
<td>79 (96)</td>
<td>60.8 (491)</td>
<td>55.2 (95)</td>
<td>54 (29)</td>
<td>57.9 (55)</td>
<td>59.3 (89)</td>
<td>56.8 (423)</td>
<td>49.4 (85)</td>
<td>61.1 (406)</td>
<td>56.8 (175)</td>
<td>55.9 (218)</td>
<td>54.1 (253)</td>
<td>67.6 (123)</td>
<td>54.8 (109)</td>
</tr>
<tr>
<td>Asthma exacerbations in past year: Asthma-related ED visit/hospital admission, % (n)*</td>
<td>14.7 (18)</td>
<td>19.0 (154)</td>
<td>13.4 (23)</td>
<td>-</td>
<td>10.5 (10)</td>
<td>8.6 (13)</td>
<td>58.3 (434)</td>
<td>58.1 (100)</td>
<td>6.0 (39/644)</td>
<td>15.6 (48)</td>
<td>76 (296)</td>
<td>44.7 (209)</td>
<td>20.3 (37)</td>
<td>35.6 (71)</td>
</tr>
<tr>
<td>Oral corticosteroid use, % (n)</td>
<td>-</td>
<td>31.7 (256)</td>
<td>47.1 (81)</td>
<td>11.1* (6)</td>
<td>36.8 (35)</td>
<td>-</td>
<td>42.3 (315)</td>
<td>77.3 (133)</td>
<td>6.5 (43)</td>
<td>43.2 (133)</td>
<td>52 (203)</td>
<td>29.3 (137)</td>
<td>20.3 (37)</td>
<td>-</td>
</tr>
<tr>
<td>BTS treatment step:</td>
<td>2, %</td>
<td>-</td>
<td>65.6</td>
<td>§</td>
<td>-</td>
<td>60</td>
<td>28.7</td>
<td>41.1</td>
<td>60.7</td>
<td>72</td>
<td>25.6</td>
<td>-</td>
<td>68.6</td>
<td>-</td>
</tr>
<tr>
<td>3, %</td>
<td>-</td>
<td>18.4</td>
<td>-</td>
<td>-</td>
<td>37.9</td>
<td>60.7</td>
<td>43.6</td>
<td>36.4</td>
<td>22.3</td>
<td>61</td>
<td>-</td>
<td>25</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>4, %</td>
<td>-</td>
<td>16.0</td>
<td>-</td>
<td>-</td>
<td>2.1</td>
<td>10.6</td>
<td>15.3</td>
<td>2.9</td>
<td>5.7</td>
<td>13.3</td>
<td>-</td>
<td>6.4</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

BTS: British Thoracic Society; ED: Emergency Department; SD: standard deviation; yrs: years. - data not available. *Patients with exacerbations were treated with oral corticosteroid or high dose inhaled corticosteroids. § CAMP is Randomized Clinical Trial of mild to moderate asthmatics and all children were on 200 μg of budesonide (ICS) plus SABA as needed. #DNA was extracted from peripheral blood samples in these studies and in the remaining DNA was extracted from saliva samples. ‘–’ no information available.
Figure 1. Forest Plots of rs7216389 for A) asthma-related hospitalizations/ED visits in thirteen studies and B) OCS/high dose ICS use in eleven studies. Odds Ratios (OR) and corresponding 95% Confidence Intervals (95% CI) for individuals with rs7216389, controlling for age, gender, and BTS treatment step.