Genetic association of the functional CDHR3 genotype with early-onset adult asthma in Japanese populations

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A R T I C L E  I N F O
Article history:
Received 18 November 2016
Received in revised form 22 January 2017
Accepted 8 February 2017
Available online 17 March 2017

Keywords:
Asthma phenotype
Cadherin-related family member 3 (CDHR3)
Early-onset asthma
Genetics
Human rhinovirus C

A B S T R A C T
Background: Recent studies have demonstrated that a coding SNP (rs6967330, Cys529→Tyr) in cadherin-related family member 3 (CDHR3), which was previously associated with wheezing illness and hospitalizations in infancy, could support efficient human rhinovirus C (RV-C) entry and replication. Here, we sought to examine the genetic contribution of this variant to the development of adult asthma.

Methods: We performed a candidate gene case–control association study of 2 independent Japanese populations (a total of 3366 adults). The odds ratios (ORs) for association of the A allele at rs6967330 with adult asthma were calculated according to age at onset of asthma. In addition, the effect of the CDHR3 variant was examined.

Results: The A allele was associated with asthma (OR = 1.56; Mantel–Haenszel p = 0.0040) when the analysis was limited to patients with early-onset adult asthma. In addition, when the analysis was limited to atopic individuals, a stronger association of the CDHR3 variant with early-onset asthma was found, and interaction of the CDHR3 genotype with atopy was demonstrated. Finally, a significant association of this variant was specifically found with a phenotype of asthma characterized by atopy, early-onset, and lower lung function.

Conclusions: Our study supports the concept that the CDHR3 variant is an important susceptibility factor for severe adult asthma in individuals who develop the disease in early life. The interaction between the CDHR3 variant and atopy indicates that genetic predisposition to early respiratory viral infection is combined with atopy in promoting asthma.

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Introduction

Asthma is a complex and heterogeneous disease with variable clinical expression over the lifespan. This variable clinical expression encompasses different clinical phenotypes and molecular endotypes depending on the age at onset, presence of allergies, frequency of exacerbations, and nature of the underlying airway inflammation. Given such phenotypic heterogeneity, selecting more homogeneous phenotypes for genetic association study could lead to identification of novel genetic factors for asthma, eventually allowing us to describe each patient by a distinctive biological mechanism or endotype and ultimately enhancing our ability to treat this complex syndrome effectively. On the basis of this concept, by limiting patients to those with specific phenotypes, we...
have identified novel genetic factors underlying particular phenotypes of asthma, including HAS2 and HCG22.

A recent genome-wide association study (GWAS) of Danish children with asthma focused on a specific asthma phenotype characterized by recurrent episodes of severe exacerbations requiring hospitalization between 2 and 6 years of age. Despite the relatively smaller sample size than those of other asthma GWASs, this GWAS of recurrent asthma exacerbations in childhood identified 1 novel susceptibility locus at the CDHR3 gene. CDHR3 is a transmembrane protein that is highly expressed in the human lung and airway epithelium. It belongs to the cadherin family of proteins, which are involved in homologous cell adhesion and several cellular processes including epithelial polarity and differentiation.

The most strongly associated variant at this locus was a non-synonymous SNP (rs6967330) that results in an amino acid change in an interdomain region of the protein (Cys529→Tyr). Introduction of the risk variant at rs6967330 by means of transfection resulted in 10-fold increased human rhinovirus C (RV-C) binding and progeny yield as compared with the nonrisk variant, providing evidence that CDHR3 is an RV-C receptor and that the asthma association signal with this functional variant in the CDHR3 gene might result from increased susceptibility to RV-C infections.

HRV-induced wheezing illnesses in early life are a significant risk factor for subsequent development of asthma. RV-C has also been reported to be the most common viral trigger of severe asthma exacerbations in children and associated with both severe disease and higher rates of hospital readmissions than other viral respiratory tract infections. Furthermore, HRV seems to be more prevalent in the airways of adolescents and young adults with asthma and a high degree of aeroallergen IgE sensitization than in controls. The presence of HRV was related to systemic eosinophilic inflammation despite ongoing treatment with inhaled corticosteroids.

In this hypothesis-driven study, therefore, we used a candidate gene case–control approach to examine the genetic contribution of the CDHR3 variant (Cys529→Tyr) in Japanese patients with adult asthma, specifically focusing on early-onset adult asthma, and to identify the specific phenotypes of adult asthma associated with increased susceptibility to RV-C infection.

Methods

Subjects

To determine the effect of the CDHR3 variant (Cys529→Tyr), we studied 2 Japanese adult asthma populations: (1) the “Tsukuba cohort,” comprising 967 healthy adults who visited the Tsukuba Medical Center for an annual health checkup and 814 asthmatic patients from the Tsukuba University Hospital and its affiliated hospitals and (2) the “Hokkaido cohort,” comprising 994 healthy adults and 591 asthmatic patients from the Hokkaido University Hospital and its affiliated hospitals.

These populations were recruited for a case–control genetic association study of asthma and atopy in Japan.

Patients were considered asthmatic on the basis of the presence of recurrent episodes of 2 or more of the 3 symptoms (coughing, wheezing, and dyspnea) associated with demonstrable reversible airflow obstruction and/or increased airway responsiveness to methacholine, as previously described. The data for age at onset of asthma were self-reported. To judge the age at onset of asthma as accurately as possible, patients were asked about episodes of dyspnea, wheezing, or cough they had experienced during childhood and puberty. In cases of uncertainty, the time of the earliest respiratory symptoms was designated as the age at onset of asthma symptoms.

The healthy adults in both populations had never been diagnosed as having asthma. Atopy was assessed by measurement of specific IgE responsiveness to 14 common inhaled allergens including Dermatophagoides farinae, grass pollens, animal dander, and molds. We defined atopy as a positive response to at least 1 of the 14 allergens.

Ethics statement

This study was approved by the Human Genome Analysis and Epidemiology Research Ethics Committee of the University of Tsukuba and by the Human Genome/Gene Analysis Research Ethics Review Committees of the Tsukuba Medical Center, RIKEN, and the Hokkaido University School of Medicine. Written informed consent was obtained from each participant before the investigation in accordance with the principles of the Declaration of Helsinki.

Genotyping

Genomic DNA was extracted from whole blood by use of an automated DNA extraction system (QuickGene-610L; Fujifilm, Tokyo, Japan). In the Tsukuba cohort, 243 patients with asthma and 967 nonasthmatic, non-COPD controls underwent GWAS genotyping. For those individuals without GWAS genotyping, genotypes for rs6967330 were defined using the TaqMan allele-specific amplification method (Applied Biosystems, Foster City, CA, USA), as described previously.

Statistical analysis

A polymorphism in rs6967330, coding SNP (G→A), converts residue cysteine to tyrosine at position 529 (Cys529→Tyr). The odds ratios (ORs) for association of the A allele with adult asthma were calculated according to the age of disease onset in the 2 independent Japanese populations. The Mantel–Haenszel method was used to estimate the pooled OR across the populations, assuming a fixed effects model. One-sided p values of less than 0.05 were judged to be significant to test whether the OR of the CDHR3 variant (Cys529→Tyr) for asthma is significantly greater than 1.

Allergic sensitizations and viral respiratory infections have long been recognized as two of the most important risk factors for exacerbations of asthma; the combination of allergic sensitization and viral illnesses greatly increases the risk of asthma exacerbation and hospitalization. We therefore examined the effect of the CDHR3 variant on early-onset adult asthma in the presence of atopy. We also examined the interaction of the CDHR3 genotype and atopy by entering the interaction term of ‘atopy * CDHR3 genotype’ into a multiple logistic regression model.

Analyzing risk factors for asthma by using an overall asthma population may be misleading and thus emphasizes the importance of careful asthma phenotyping in epidemiology studies, which will have important implications for understanding the pathogenesis of asthma. We therefore searched for phenotype–genotype correlations; we examined the effect of the CDHR3 genotype on the development of previously identified clusters of adult asthma by using multinomial logistic regression analysis.

In our previous study, 2-step cluster analysis of 880 Japanese adult asthma patients identified 6 phenotypes using 8 variables: age, sex, age at onset of the disease, smoking status, total serum IgE, %FEV1, FEV1/FVC, and specific IgE responsiveness to common inhaled allergens. The 6 phenotypes were cluster A: older age at onset, no airflow obstruction; cluster B: childhood onset, normal-to-mild airflow obstruction; cluster C: childhood onset, the longest disease duration, and moderate-to-severe airflow obstruction; cluster D: older age at onset, severe airflow obstruction; cluster E: middle age at onset, no airflow obstruction; and cluster F: older age at onset, mild-to-moderate airflow obstruction. The 2 strongest
discriminatory variables for assignment of these clusters were age at onset of asthma and %FEV₁. Using these 2 variables, we developed a CART model, which we used to determine the cluster for each patient with adult asthma in the current study. We used 1082 patients with adult asthma for whom data on %FEV₁ were available. In the combined analysis was limited to patients with early-onset adult asthma (age < 10 years). In the current study, we have shown the importance of the functional CDHR3 polymorphism in the pathogenesis of early-onset asthma beyond racial diversity. Although the replication of previously published GWAS findings may lack the impact of new discoveries, our finding is still important in its own right, especially given that recent GWAS of childhood asthma failed to replicate the genetic effect of CDHR3 in a Dutch population. Our meta-analysis of 2 independent Japanese populations of adult asthma showed that, compared with wild-type CDHR3, the CDHR3 variant (Cys529→Tyr, rs6967330) is significantly associated with the development of early-onset adult asthma. CDHR3 is an epithelial cell-binding site for HRV-C; the CDHR3 gene variant associated with increased asthma hospitalization risk conferred increased airway epithelial expression of CDHR3 and large increases in HRV-C

### Results

The characteristics of the Tsukuba cohort (N = 967) and of the Hokkaido cohort (N = 994) are shown in Table 1. The rs6967330 of the control groups of both cohorts did not deviate from the Hardy–Weinberg equilibrium (p > 0.5). In both the Tsukuba and the Hokkaido cohorts, the greater number of the A allele at rs6967330, which corresponds to the CDHR3 variant (Cys529→Tyr), was found in patients with asthma (OR = 1.52 and 1.61; p = 0.036 and 0.026, respectively) when the analysis was limited to patients with early-onset adult asthma (age at onset of the disease, 10 years or younger). In the combined analysis of the Tsukuba and Hokkaido cohorts, the presence of the A allele was associated with the development of early-onset asthma with an OR (Mantel–Haenszel p value) of 1.56 (0.0040) (Table 2, Fig. 1). When the analysis was further limited to individuals with atopy in the combined population, the stronger association of the CDHR3 variant with early-onset asthma was found in atopic individuals (OR for the A allele = 2.02 [1.41–2.88]; p = 0.00015; Table 2). The interaction of the CDHR3 genotype with atopy was demonstrated in the regression analysis by formulating product variables of the CDHR3 genotype and atopy in the model (p for interaction = 0.027). In contrast, atopy was not associated with the CDHR3 variant (OR for the A allele = 0.93 [0.77–1.10]; Mantel–Haenszel p = 0.41).

When the association of this CDHR3 genotype with specific asthma phenotypes was evaluated by multinomial logistic regression analysis according to each of the 6 clusters identified in this study, significant associations of the variant were found with cluster C (OR 1.95, p = 0.0037; Table 3), which is characterized by childhood onset, longest disease duration, and moderate-to-severe airflow obstruction.

### Discussion

In the current study, we have shown the importance of the functional CDHR3 polymorphism in the pathogenesis of early-onset asthma beyond racial diversity. Although the replication of previously published GWAS findings may lack the impact of new discoveries, our finding is still important in its own right, especially given that recent GWAS of childhood asthma failed to replicate the genetic effect of CDHR3 in a Dutch population. Our meta-analysis of 2 independent Japanese populations of adult asthma showed that, compared with wild-type CDHR3, the CDHR3 variant (Cys529→Tyr, rs6967330) is significantly associated with the development of early-onset adult asthma. CDHR3 is an epithelial cell-binding site for HRV-C; the CDHR3 gene variant associated with increased asthma hospitalization risk conferred increased airway epithelial expression of CDHR3 and large increases in HRV-C

### Table 1

Characteristics of the study populations.

<table>
<thead>
<tr>
<th></th>
<th>Tsukuba cohort</th>
<th>Hokkaido cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy controls</td>
<td>Asthmatic patients</td>
</tr>
<tr>
<td>Number</td>
<td>967</td>
<td>751</td>
</tr>
<tr>
<td>Sex (female, %)</td>
<td>526 (54.4)</td>
<td>466 (57.2)</td>
</tr>
<tr>
<td>Age, y (range)</td>
<td>50.0 (27–74)</td>
<td>56.3 (19–100)</td>
</tr>
<tr>
<td>Age at asthma onset</td>
<td>40.7 (0–88)</td>
<td></td>
</tr>
<tr>
<td>Smoking pack-year (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–10</td>
<td>697 (62.8)</td>
<td>514 (65.4)</td>
</tr>
<tr>
<td>&gt;10</td>
<td>127 (13.1)</td>
<td>93 (11.8)</td>
</tr>
<tr>
<td>Atopy (%)</td>
<td>541 (55.9)</td>
<td>397 (70.9)</td>
</tr>
<tr>
<td>FEV₁ expred. (% SD)</td>
<td>85.6 (22.5)</td>
<td>88.7 (27.3)</td>
</tr>
<tr>
<td>FEV₁/FVC (%)</td>
<td>71.2 (12.2)</td>
<td>68.4 (13.2)</td>
</tr>
</tbody>
</table>

In the Tsukuba cohort, information on smoking and atopy was missing in 28 and 254 patients with asthma, respectively. In the Hokkaido cohort, information on smoking and atopy was missing in 29 and 8 patients with asthma, respectively. In the Tsukuba and Hokkaido cohorts, information on FEV₁ was missing in 229 and 47 patients with asthma, respectively.

1. Atopy was defined as the presence of specific IgE Ab in at least 1 of 14 common inhaled allergens.

### Table 2

Association of the CDHR3 SNP (rs6967330) with early-onset asthma.

<table>
<thead>
<tr>
<th></th>
<th>Tsukuba cohort</th>
<th>Hokkaido cohort</th>
<th>Meta-analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>G</td>
<td>Total</td>
</tr>
<tr>
<td>All participants</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>144</td>
<td>1790</td>
<td>1934</td>
</tr>
<tr>
<td>Patients with early-onset asthma (onset at ≥10 years)</td>
<td>30</td>
<td>246</td>
<td>276</td>
</tr>
<tr>
<td>Total</td>
<td>174</td>
<td>2036</td>
<td>2210</td>
</tr>
<tr>
<td>Atopic individuals</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>68</td>
<td>1014</td>
<td>1082</td>
</tr>
<tr>
<td>Patients with early-onset asthma (onset at ≥10 years)</td>
<td>20</td>
<td>180</td>
<td>200</td>
</tr>
<tr>
<td>Total</td>
<td>88</td>
<td>1194</td>
<td>1282</td>
</tr>
</tbody>
</table>

1. One-sided p values of less than 0.05 were judged to be significant to test if the OR of the A allele for asthma is significantly greater than 1.
binding and replication.\(^9\) Therefore, our study has identified the HRV\(\text{CDHR3}\) pathway as a distinct molecular mechanism or endotype underlying early-onset allergic adult asthma.

Clustering of asthma patients based on age at onset and lung function indicated that the \(\text{CDHR3}\) variant may be specifically responsible for the development of a particular phenotype of asthma characterized by early onset, atopy, and severe airflow obstruction. In fact, a study of a high risk birth cohort showed that among outpatient viral wheezing illnesses in early childhood, those caused by RV infections were the most significant predictors of decreased lung function up to the age of 8 years, although whether low lung function is a cause and/or effect of RV infections remains to be determined.\(^{12}\) Rhinovirus is also found in the sputum or pharyngeal swab specimens of asthmatic adults without concurrent symptoms of infection or asthma exacerbation; positivity is associated with lower lung function and more frequent asthma symptoms.\(^{24}\) Accordingly, although records on the early life history of severe wheeze and asthma exacerbation were not available for the patients in our cohort, severe wheezing illnesses or frequent asthma exacerbations related to the \(\text{CDHR3}\) variant may have caused airway injury, thus chronically diminishing lung function. Our findings in adult populations together with the original study in a pediatric population may, therefore, indicate that the genetic effect of \(\text{CDHR3}\) underlies asthma persistence from childhood to adulthood.

We identified a much stronger effect of the \(\text{CDHR3}\) variant in atopic individuals. RV infections and allergy in early life synergize to increase the risk of developing asthma,\(^{25}\) which clearly involves several mechanisms. In a study of human subjects infected with rhinovirus, an increase in the level of IL-33 was detected in the bronchoalveolar lavage fluid;\(^{30}\) the levels of IL-5 and IL-13 secreted by both cultured T cells and ILC2s were increased in the supernatants of rhinovirus-infected airway epithelial cells. RV-induced lower airway inflammation also precipitates asthma exacerbations through impaired Th1/IL-10 and augmented Th2 responses.\(^{27}\) By enhancing type-2 inflammation, therefore, repeated severe RV infections may accelerate the progression toward expression of persistent aggravation of airway disease in asthma-like manifestations. In addition, allergic airway inflammation in asthma can impair epithelial barrier function, and \textit{in vitro} studies have indicated that RV replication is enhanced when the apical cells of well-differentiated epithelial cell cultures are either damaged or stripped away.\(^{28}\) This could secondarily increase the accessibility of \(\text{CDHR3}\) for RV-C binding in individuals with respiratory allergy. As a result, RV-C could infect more cells, leading to severe respiratory illness.\(^{29}\) Finally, a mechanism through which IgE impairs rhinovirus immunity and underlies asthma exacerbations was also demonstrated\(^{10}\); the Preventative Omalizumab or Step-Up Therapy for Fall Exacerbations (PROSE) study compared the effects of initiating omalizumab, ICS boost, or placebo before the fall season in inner-city children and adolescents with asthma and found that IFN-\(\alpha\) responses to rhinovirus were significantly increased within the omalizumab-treated group. Therefore, IgE likely plays an important role in the promotion of rhinovirus infection progression to exacerbations by generating an inflammatory milieu. Altogether, these mechanisms may underlie our finding of a significant effect of the interaction between the \(\text{CDHR3}\) variant and atopy on the development of early-onset asthma.

In the current study, the functional \(\text{CDHR3}\) genotype was specifically associated with atopic early-onset asthma with decreased lung function. On the basis of many studies including the current one, several endotypes of severe asthma have been described, including increased susceptibility to viral infections, increased bacterial colonization, impaired lung development, and enhanced type-2 inflammation.\(^{31}\) Given such a heterogeneity of severe asthma, a better understanding of the role of HRV in the pathogenesis of the disease will pave the way to treating patients on the basis of these endotypes as indicated by the appropriate biomarkers or treatable traits corresponding to each individual endotype.

### Table 3
Multinomial logistic regression between the \(\text{CDHR3}\) SNP (rs6967330) and the asthma clusters.

<table>
<thead>
<tr>
<th>Cluster</th>
<th>Phenotype</th>
<th>(\text{CDHR3}) genotype</th>
<th>(p) Value</th>
<th>Odds ratio (95% CI)</th>
<th>Sex (female, %)</th>
<th>Age (y, range)</th>
<th>Age of asthma onset (y, SD)</th>
<th>FEV1 Spredicted (SD)</th>
<th>Atopy (n, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clusters A</td>
<td>Late-onset Mild Less-atopic</td>
<td>AA/AG (%)</td>
<td>156 (82.5)</td>
<td>189</td>
<td>0.58</td>
<td>1.12 (0.75–1.66)</td>
<td>120 (63.5)</td>
<td>63.7 (31–84)</td>
<td>54.6 (12.0)</td>
</tr>
<tr>
<td>Cluster B</td>
<td>Early-onset Mild Less-atopic</td>
<td>AA/AG (%)</td>
<td>166 (80.6)</td>
<td>206</td>
<td>0.20</td>
<td>1.27 (0.88–1.84)</td>
<td>105 (51.0)</td>
<td>37.5 (18–83)</td>
<td>10.1 (8.4)</td>
</tr>
<tr>
<td>Cluster C</td>
<td>Moderate-to-severe Severe</td>
<td>AA/AG (%)</td>
<td>76 (73.1)</td>
<td>104</td>
<td>0.37</td>
<td>1.95 (1.24–3.05)</td>
<td>41 (40.2)</td>
<td>43.1 (19–82)</td>
<td>14.9 (10.3)</td>
</tr>
<tr>
<td>Cluster D</td>
<td>Late-onset Severe</td>
<td>AA/AG (%)</td>
<td>88 (77.9)</td>
<td>113</td>
<td>0.084</td>
<td>1.50 (0.95–2.38)</td>
<td>63 (56.3)</td>
<td>64.8 (38–84)</td>
<td>50.3 (13.6)</td>
</tr>
<tr>
<td>Cluster E</td>
<td>Middle-age onset Female-dominant</td>
<td>AA/AG (%)</td>
<td>182 (86.7)</td>
<td>210</td>
<td>0.33</td>
<td>0.81 (0.54–1.23)</td>
<td>136 (64.8)</td>
<td>52.5 (21–82)</td>
<td>41.1 (13.8)</td>
</tr>
<tr>
<td>Cluster F</td>
<td>Late-onset Less-atopic</td>
<td>AA/AG (%)</td>
<td>223 (85.8)</td>
<td>260</td>
<td>0.49</td>
<td>0.88 (0.61–1.27)</td>
<td>165 (63.7)</td>
<td>62.4 (36–88)</td>
<td>51.7 (11.6)</td>
</tr>
</tbody>
</table>

\(^1\) Owing to the low frequency of the homozygote for the A allele, this group was combined with the heterozygote for these analyses.
Acknowledgements

We thank all the physicians who participated in recruiting the study participants and collecting the data. We also thank Ms F. Miyamasu, an associate professor at the Faculty of Medicine, University of Tsukuba, who proofread and commented on this paper. Ms Takako Nakamura gave technical assistance with DNA extraction from the clinical samples.

Conflict of interest

NH has received lecture fees from AstraZeneca, Astellas Pharma and Boehringer Ingelheim. The rest of the authors have no conflict of interest.

Authors’ contributions

JK analyzed the data and drafted the manuscript. HM, YY, HY, HI, TSak, and NH contributed to the design and data analyses and drafted the manuscript. YK, HK, TN, TSai, EN, SK, MN, TH, and MT were involved in the study conception and data interpretation. NH supervised the entire research. All authors read and approved the final manuscript.

References