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Original article

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

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CDHR3, cadherin-related family member 3; RV-C, human rhinovirus C; ORs, odds ratios; GWAS, genome-wide association study; HAS2, hyaluronan synthase 2; HCG22, HLA complex group 22; CART, classification and regression trees; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity

ABSTRACT

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* genotype on the development of specific asthma phenotypes 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.

Introduction

Asthma is a complex and heterogeneous disease with variable clinical expression over the lifespan.[1] 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.[2] 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

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have identified novel genetic factors underlying particular phenotypes of asthma, including *HAS2*[3] and *HCG22*.[4]

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.[5] 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.[6,7] It belongs to the cadherin family of proteins, which are involved in homologous cell adhesion and several cellular processes including epithelial polarity and differentiation.[8] The most strongly associated variant at this locus was a nonsynonymous SNP (rs6967330) that results in an amino acid change in an interdomain region of the protein (Cys529→Tyr).[9] 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 strong 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.[10] RV-C has also been reported to be the most common viral trigger of severe asthma exacerbations in children and associated with both severer disease and higher rates of hospital readmissions than are other viral respiratory tract infections.[11–13] 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.[14] 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,[3,15] and (2) the "Hokkaido cohort," comprising 994 healthy adults and 591 asthmatic patients from the Hokkaido University Hospital and its affiliated hospitals.[15–17] These populations were recruited for a case–control genetic association study of asthma and atopy in Japan.[18]

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.[19] 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.[19] 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.[17]

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; Fuji film, Tokyo, Japan). In the Tsukuba cohort, 243 patients with asthma and 967 nonasthmatic, non-COPD controls underwent GWAS genotyping.[3] 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.[20]

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.[21] 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, %FEV_1, FEV_1/FVC, and specific IgE responsiveness to common inhaled allergens.[17] 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