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Association of genetic variation in chitotriosidase with atopy in Korean children

Kyung Won Kim, MD, PhD^{*}; Joonhee Park, MSc^{†,‡,§}; Ji Hyun Lee, PhD^{‡,§,¶}; Hee Seon Lee, MD^{*}; Jaechun Lee, MD[#]; Keun-Hwa Lee, PhD[#]; Kyung Eun Lee, PhD^{*,||}; Jung Yeon Hong, MSc^{*,†}; Mi Na Kim, MSc^{*,†}; Won Il Heo^{*,†}; Min Goo Lee, MD, PhD^{*,‡,§,¶,||}; Myung Hyun Sohn, MD, PhD^{*,†,¶,||}; and Kyu-Earn Kim, MD, PhD^{*,†}

^{*} Department of Pediatrics and Institute of Allergy, Yonsei University College of Medicine, Seoul, Korea

[†] Brain Korea 21 Project for Medical Sciences, Yonsei University College of Medicine, Seoul, Korea

[‡] Department of Pharmacology, Yonsei University College of Medicine, Seoul, Korea

[§] Pharmacogenomic Research Center for Membrane Transporters, Yonsei University College of Medicine, Seoul, Korea

[¶] Research Center for Human Natural Defense System, Yonsei University College of Medicine, Seoul, Korea

^{||} Severance Biomedical Science Institute, Yonsei University College of Medicine, Seoul, Korea

[#] Environmental Health Center, School of Medicine, Jeju National University, Jeju, Korea

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ABSTRACT

Background: The atopic diseases, which are the most common chronic diseases of childhood, are complex genetic diseases that involve the contribution of multiple genetic factors to disease pathophysiology. Chitotriosidase is involved in innate immunity, but the association of chitotriosidase with allergic diseases remains unclear.

Objective: To examine the contribution of genetic variation of the chitotriosidase-encoding gene *CHIT1* to atopic phenotypes in a Korean cohort of children.

Methods: We identified *CHIT1* variations in a Korean population and conducted association analyses using 295 atopic and 242 nonatopic children. An independent replication study was performed using DNA samples from 148 atopic and 243 nonatopic children. All children were unrelated. We performed Western blot analysis in each genotype in vitro to see whether the *CHIT1* A442G variation affects the final protein expression levels.

Results: In the case-control association analysis, atopy was significantly associated with a single A442G (rs1065761) polymorphism in *CHIT1* (odds ratio = 1.32, $P = .01$). Children with the c.442G risk allele had significantly higher blood eosinophils ($P = .001$), total serum IgE ($P = .007$), and eosinophil cationic protein ($P = .02$) levels. The results of the replication stage analysis confirmed a significant association between the A442G polymorphism and childhood atopy. The joint analysis of the exploratory and replication studies displayed a stronger significant association. The relative protein expression levels of chitotriosidase were significantly higher in both cell lysate and media with the G transfection compared with the wild type.

Conclusion: These results indicate that the nonsynonymous A442G polymorphism in *CHIT1* is associated with risk of atopy.

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Reprints: Myung Hyun Sohn, MD, PhD, Department of Pediatrics, Yonsei University College of Medicine, 50 Yonsei-ro, Seodaemun-gu, Seoul, 120-752, Republic of Korea; E-mail: mhsohn@yuhs.ac.

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Introduction

Atopy, which is an essential immune phenotype involved in orchestration of various cells and mediators, is reportedly a risk factor for asthma and other allergic diseases.¹ Many researchers have sought to elucidate the pathogenesis of atopy, namely, allergic immune reactions. Although these studies have provided a glimpse of the complexity of allergic responses, the cellular and molecular events in innate and adaptive responses are not yet fully understood.²

Chitinases are hydrolases that can cleave the environmentally abundant chitin polymer. These enzymes inhibit chitin-induced innate immune and injury responses, enhance adaptive immune responses, especially T_H2 responses, and contribute to healing and

fibrosis.³ Human chitinases include 2 true chitinases, chitotriosidase and acid mammalian chitinase (AMCase), and a chitinase-like protein, including YKL-40 (also known as human cartilage glycoprotein 39 or chitinase 3–like 1 [CH13L1]). AMCase also contributes to T_H2 inflammation by controlling innate responses and augmenting the evolution of adaptive immunity.⁴ Polymorphisms in the *CHIA* gene, which encodes AMCase, have been found to be associated with asthma.⁵ We recently reported that genetic variation in the promoter region of the *CH13L1* gene that encodes YKL-40 functionally regulates the expression of YKL and is associated with high levels of IgE.⁶

Chitotriosidase, 1 of 18 glycosylhydrolase family members, is the major chitinase in human respiratory tissues, whereas AMCase is the major chitinase in lungs of mice.³ Chitotriosidase, which is a component of the innate immune system, may play a role in defense against chitin-containing fungal, filarial, or gram-negative bacterial infection.^{7–9} Although serum chitotriosidase levels were increased in asthma patients in recent studies, chitotriosidase activity in bronchoalveolar lavage was not increased and tended to be decreased in patients with asthma in another recent conflicting study.^{10–12} Furthermore, the association of genetic variants of *CHIT1*, which encodes chitotriosidase, with asthma is also controversial.^{10,13} More recently, additional studies have suggested that human chitinases and their genetic variations are associated with asthma or atopy.^{5,14,15} Indeed, one recent study demonstrated that the interaction between the *CHIT1* gene and the environment was associated with severe asthma.¹⁶ Furthermore, the gene distribution of the human *CHIT1* gene has been reported to vary among different ethnic groups.¹⁷ Overall, our collective understanding of the role of chitotriosidase in atopy is still quite limited. Therefore, we conducted an association study of *CHIT1* polymorphisms with respect to susceptibility to atopy in Korean children.

Methods

Study Participants

In the initial exploratory stage of this study, 295 unrelated atopic children were recruited from the Severance Hospital of Yonsei University in Seoul, Korea. Diagnosis of atopy was made based on either a positive skin test result to more than 1 of the 12 common local aeroallergens (Allergopharma, Reinbek, Germany) or serum total IgE concentrations above 100 IU/mL. In addition, 242 unrelated, nonatopic children were age-matched healthy control children who visited the hospital for general health workups. The nonatopic children included in this group were required to have a skin prick test result that was negative for the common aeroallergens and serum total IgE concentrations less than 100 IU/mL without taking any medication. Detailed information for atopic and nonatopic children is given in Table 1. Asthma was defined as the presence of recurrent wheezing or coughing in the absence of a cold in the preceding 12 months with a physician's diagnosis (at any time in the patient's history), bronchial hyperresponsiveness on methacholine challenge (provocation concentration that caused a decrease in forced expiratory volume in 1 second [FEV₁] of 20%, ≤ 16 mg/mL), or at least 12% reversibility of the FEV₁ after inhalation of a β_2 -agonist as per the criteria of the American Thoracic Society.¹⁸

For the replication stage, a total of 469 independent, unrelated children, including 202 atopic and 267 nonatopic children, were recruited from 2 elementary schools in Jeju, Korea. The study included administering a survey, measuring serum total IgE levels, and performing skin prick tests. We used a modified International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire¹⁹ to determine the prevalence of symptoms and to diagnose allergic diseases. Atopy was defined as a positive skin test result on exposure to one or more extracts of *Dermatophagoides pteronyssinus* and

Table 1

Baseline characteristics of the study participants

Characteristic	Atopic	Nonatopic	P value ^a
No. (%) of patients	295 (54.9)	242 (45.1)	
Age, mean (SD), y	9.74 (2.89)	8.79 (3.06)	.003
Male sex, No. (%)	195 (66.1)	136 (56.7)	.03
Total serum IgE levels, mean (SEM), IU/L	618.68 (38.37)	49.52 (2.66)	<.001
Serum ECP, mean (SEM), μ g/L	26.51 (1.74)	15.04 (1.14)	<.001
Total blood eosinophils, mean (SEM), μ L	430.61 (19.19)	197.17 (11.38)	<.001
Asthma, No. (%)	144 (48.8)	82 (34.2)	.001

Abbreviations: ECP, eosinophil cationic protein.

^a χ^2 test or *t* test was used as appropriate.

Dermatophagoides farinae or serum total IgE concentrations above 100 IU/mL in the children with a history of asthma or allergic rhinitis. The nonatopic controls were included in this group if they met the following conditions: a negative skin prick test result and serum total IgE concentrations less than 100 IU/mL in children without a history of respiratory symptoms or allergy. All study participants were provided written informed consent to participate in the study according to the appropriate institutional review board.

Gene Scanning and Selection of *CHIT1* Polymorphisms for Genotyping

To identify *CHIT1* polymorphisms prevalent in the Korean population, gene scanning was performed on the 48 healthy children. The promoter region at -2.0 Kb from the translation initiation site, the coding exons, and the flanking intronic sequences of the *CHIT1* gene were amplified in 15 polymerase chain reaction fragments and sequenced by an automated genetic analyzer (model 3700; Applied Biosystems, Foster City, California). Haplotype analysis was performed using the Haploview 3.2 program based on a standard expectation-maximization algorithm to construct haplotype blocks.²⁰ For genotyping in individual children, we selected 3 single-nucleotide polymorphisms (SNPs) of *CHIT1* with a minor allele frequency of greater than 5% in the screening stage. Genotype screening of each sample was performed by the SNaPshot or SNaPIT method (Applied Biosystems) according to protocols supplied by the manufacturer.

Measurements of Blood Eosinophil Count, Serum Eosinophil Cationic Protein, and Serum Total IgE

Peripheral venous blood was collected from all study participants. An eosinophil count was performed on an aliquot of whole venous blood using the NE-8000 system (Sysmex, Kobe, Japan). The serum fraction was then separated from whole blood, and eosinophil cationic protein (ECP) and serum total IgE levels were measured using the CAP system FEIA (Pharmacia, Diagnostics, Uppsala, Sweden).

Skin Prick Test

Skin prick tests were performed with 12 common allergens, including 2 types of house dust mites, cat and dog epithelia, and mold and pollen antigens (Allergopharma, Reinbek, Germany). Negative and positive controls were saline and 0.5% histamine hydrochloride, respectively.

Western Blot Analysis of Chitotriosidase in HEK293 Cells

HEK293 cells were maintained in Dulbecco modified Eagle medium-HG (Invitrogen, Carlsbad, California) supplemented with 10% fetal bovine serum. Plasmids were transiently transfected into HEK293 cells using Lipofectamine Plus Reagent (Invitrogen). For

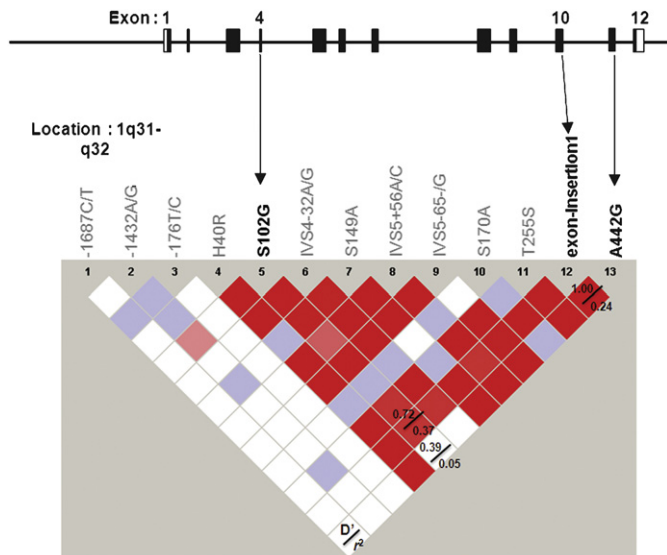


Figure 1. Linkage disequilibrium (LD) structure of the *CHIT1* gene in the Korean population. The upper diagram shows the genomic structure of *CHIT1*, and the lower diagram shows the LD (D'/r^2) among single-nucleotide polymorphisms (SNPs) in this study. The arrow indicates the SNPs selected for the association analyses. Gray letters represent SNPs excluded from genotype analyses due to an allele frequency less than 0.05.

plasmids, the *CHIT1* clone was subcloned into pcDNA3.1(-) at the *NheI/KpnI* sites to generate pcDNA3.1(-)-wt-*CHIT1*. *CHIT1* A442G was made by generating a single mutation at the c.1325C>G site of *CHIT1* using a polymerase chain reaction–based mutagenesis technique. For Western blotting, cell lysates and media were loaded and separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. The separated proteins were transferred and immobilized onto a nitrocellulose membrane and immunoblotted with chitotriosidase antibody (Santa Cruz Biotechnology, Santa Cruz, California), aldolase (Santa Cruz Biotechnology), and neomycin antibody, respectively. Protein bands were detected by enhanced chemiluminescence (Amersham Biosciences, Amersham, England).

Statistical Analysis

Statistical analyses were performed using SPSS statistical software, version 11.5 (SPSS Inc, Chicago, Illinois), and Graphpad Prism 4.0 (Graphpad Software Inc, San Diego, California) software packages. Genotype frequency comparisons between atopic and nonatopic groups were performed by χ^2 tests. The Fisher exact test was used if expected cell frequencies were lower than 5. Adjustment for

multiple tests was made by multiplying the P value by the number of SNPs being investigated (Bonferroni correction). Genotype frequencies at each SNP were tested for adherence to the Hardy-Weinberg equilibrium.

The results of multiple measurements are presented as mean (SEM). Serum IgE level and total eosinophil count were not normally distributed in arithmetic scale and were thus log-transformed (normality verified by the Kolmogorov-Smirnov test) before being used for statistical comparisons. Statistical analysis was performed with the Kruskal-Wallis test or analysis of variance followed by the Mann-Whitney test, as appropriate. All P values were based on 2-sided comparisons, and $P < .05$ was considered statistically significant.

Results

Demographics and Clinical Characteristics of the Study Participants

Baseline characteristics of the 295 atopic and 242 nonatopic children in the initial exploratory stage of the study are summarized in Table 1. The atopic group had a slightly higher male-to-female ratio ($P = .03$), and the children were older ($P = .003$) compared with the nonatopic group. Atopic children had significantly higher total serum IgE, ECP, and blood eosinophil levels, which are well-recognized complications of atopy ($P < .001$). The rate of asthma was higher in the atopic group compared with the nonatopic group ($P = .001$). The baseline characteristics of the 469 independent participants, including 202 atopic and 267 nonatopic children, in the replication stage of the study are summarized in eTable 1.

CHIT1 Genetic Variations in the Korean Population

To identify *CHIT1* variations in the Korean population, we performed direct DNA sequencing of the *CHIT1* gene in genomic DNA samples derived from 24 atopic and 24 nonatopic children. Although nearly 334 SNPs have been identified in the human *CHIT1* gene (www.genecards.org) to date, 13 genetic variations with an allele frequency of more than 5% were discovered after comprehensive gene scanning. All *CHIT1* exons and exon-intron junctions were sequenced, including -2.0 Kb from the translation initiation site. The linkage disequilibrium structure of the *CHIT1* gene is shown in Figure 1. Of the 13 variations, only 3 genetic variations were selected for the *CHIT1* association study after considering linkage based on an r^2 threshold greater than 0.8 (Fig 1).

Association of *CHIT1* Polymorphisms and Susceptibility to Atopy

To evaluate whether *CHIT1* genetic variations are associated with susceptibility to atopy, we performed case-control association studies of the 3 SNPs in all 537 children (Table 2). The genotype

Table 2
Frequency of *CHIT1* genetic variations in atopic and nonatopic patients

SNP (gene type)	Group, No. (%)		P value [OR (95% CI)]			
	Atopic	Nonatopic	Dominant	Recessive	Codominant	Allele
S102G (rs2297950)						
G/G	145 (49.2)	123 (51)	.76 [1.03 (0.87-1.22)]	.39 [1.14 (0.84-1.56)]	.54	.46
G/A	120 (40.7)	99 (41.1)				
A/A	30 (10.2)	19 (7.9)				
Exon-insertion (rs3831317)						
INS/INS	93 (32.4)	87 (36.1)	.40 [1.08 (0.9-1.3)]	.34 [1.12 (0.88-1.43)]	.27	.29
INS/-	144 (50.2)	119 (49.4)				
-/-	50 (17.4)	35 (14.5)				
A442G (rs1065761)						
CC	221 (75.4)	202 (84.2)	.01 [1.32 (1.06-1.65)]		.02	.03
CG	72 (24.6)	37 (15.4)				
GG	0 (0)	1 (0.4)				

Abbreviations: CI, confidence interval; OR, odds ratio; SNP, single-nucleotide polymorphism.

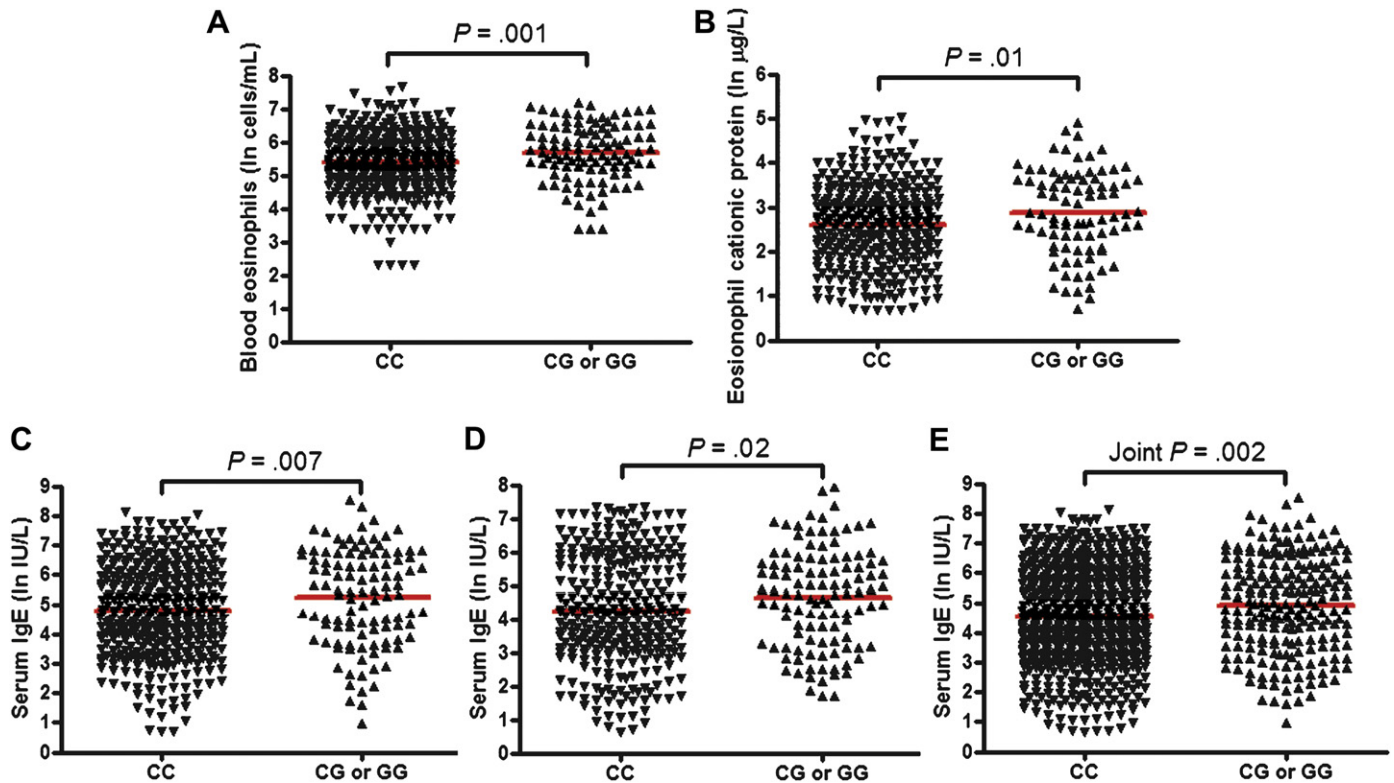


Figure 2. Total eosinophil count (TEC), serum eosinophil cationic protein (ECP), and IgE levels according to *CHIT1* A442G (c.1325C>G, rs1065761) genotype. Scatterplot illustrating TEC (a) and serum ECP (b) in the exploratory study and serum total IgE levels in the exploratory study (c), replication study (d), and joint analysis (e) for each genotype. The y-axis represents log-transformed TEC, serum ECP, and total IgE values, and horizontal bars in the scatterplots represent mean values.

distributions of the SNPs did not deviate from Hardy-Weinberg equilibrium ($P > .05$) in each set of samples. Of the 3 SNPs tested, only 1 variation showed a significant association with atopy. The A442G (c.1325C>G, rs1065761) had P values of .01 in risk allele dominant mode (odds ratio, 1.32; 95% confidence interval, 1.06–1.65), .02 in codominant mode, and .03 in allele frequency. The S102G (c.304G>A, rs2297950) had a P value of .76 in risk allele dominant mode, .39 in recessive mode, .54 in codominant mode, and .46 in allele frequency. The exon-insertion1 (rs3831317), which is a 24–base pair (bp) duplication, had a P value of .40 in risk allele

dominant mode, .34 in recessive mode, .27 in codominant mode, and .29 in allele frequency. The P value of A442G obtained in risk allele dominant mode remained significant after conservative Bonferroni correction for multiple testing ($\alpha/\text{number of comparison} = .05/3$).

Association of *CHIT1* Polymorphisms With Blood Eosinophil Count, Serum Total IgE Level, and ECP Level

Because *CHIT1* A442G was found to be associated with atopy, blood eosinophil counts, serum IgE levels, and ECP levels were

Table 3
Association results for *CHIT1* A442G (rs1065761) and childhood atopy^a

Variant	Exploratory stage		Replication stage	
	Atopic	Non-atopic	Atopic	Non-atopic
Genotypes, N of individuals (%)				
CC	221 (75.4)	202 (84.2)	123 (61.8)	207 (79.3)
CG	72 (24.6)	37 (15.4)	72 (36.2)	52 (19.9)
GG	0 (0)	1 (0.4)	4 (2.0)	2 (0.8)
Total	293 (100)	240 (100)	199 (100)	261 (100)
Alleles, N of chromosomes (%)				
C	514 (87.7)	441 (91.9)	318 (79.9)	466 (89.3)
G	72 (12.3)	39 (8.1)	80 (20.1)	56 (10.7)
Total	586 (100)	480 (100)	398 (100)	522 (100)
*P _{genotype} in codominant mode, [P _{CC&CG} comparison, OR (95% CI)]				
	0.019 [0.011, 1.79 (1.45–2.79)]		0.001 [0.0003, 2.34 (1.54–3.57)]	
*Joint P _{genotype} 0.00009 [0.00002, 1.94 (1.43–2.63)]				
*P _{genotype} in minor allele dominant mode, OR (95% CI)				
	0.014, 1.32(1.06–1.65)		0.0002, 2.38 (1.57–3.60)	
*Joint P _{genotype} 0.00002, 1.92 (1.42–2.59)				
P _{allele} , OR (95% CI)				
	0.027, 1.25 (1.02–1.54)		0.0001, 2.10 (1.45–3.04)	
*Joint P _{allele} 0.00006, 1.32 (1.15–1.51)				

Abbreviations: CI, confidence interval; OR, odds ratio.

^a P values were obtained by comparing atopic and nonatopic children using the χ^2 test or Fisher exact test (expected cell value <5) in each mode.

examined in the children. Higher peripheral blood eosinophil counts and serum total IgE levels are strong risk factors for subsequent development of atopy. Figure 2 shows log-transformed blood eosinophil counts, serum ECP levels, and total IgE values. Blood eosinophil counts were higher for individuals with the CG or GG genotype (mean [SE], 5.69 [0.09] ln cells/ μ L) than those with the CC genotype (5.37 [0.04] cells/ μ L, $P = .001$, Fig 2A). Serum ECP levels were also higher in children with the CG or GG genotype than in those with the CC genotype (2.88 [0.10] vs 2.60 [0.05] ln μ g/L, $P = .02$, Fig 2B). Serum total IgE was higher in children with the CG or GG genotype than in children with the CC genotype (5.25 [0.16] vs 4.79 [0.08] ln IU/L, $P = .007$, Fig 2C).

Association of *CHIT1* Polymorphisms With Susceptibility to Atopy in Another Independent Cohort

Because *CHIT1* A442G had an association with atopy, this SNP was genotyped in an independent cohort to reevaluate this association (Table 3). Importantly, this replication stage analysis also demonstrated a significant association between A442G and childhood atopy (genotypic $P = .001$ in codominant mode, $P < .001$ in minor allele dominant mode, and $P < .001$ in allelic frequency). Moreover, the joint analysis of the exploratory and replication studies that included 492 atopic and 501 nonatopic children displayed a much more significant association between the *CHIT1* A442G (c.1325C>G, rs1065761) polymorphism and atopy (genotypic $P < .001$ in codominant mode, $P < .001$ in minor allele dominant mode, and allelic $P < .001$).

In addition, in the replication stage analysis, serum total IgE was higher in children with the CG or GG genotype than in those with the CC genotype (4.66 [0.14] vs 4.26 [0.09] ln IU/L, $P = .02$, Fig 2D). The joint analysis of the exploratory and replication studies also displayed a much more significant association between the CG or GG genotypes and serum total IgE levels (joint $P = .002$, Fig 2E).

Protein Expression of the *CHIT1* A442G Variant

To investigate the effect of A442G variant on the protein expression of chitotriosidase in vitro, HEK293 cells were transfected with wild-type and A442G mutant clone of *CHIT1*, respectively. Aldolase and neomycin were blotted for confirming same loaded protein and transfected clones, respectively. In the Western blot analysis, the relative protein expression levels of chitotriosidase were significantly higher in A442G mutant transfected cell lysate compared with wild type. According to increased protein expression, the amounts of secreted proteins in media were higher A442G mutant transfected cells than wild type (eFig 1).

Discussion

Chitotriosidase, and not AMCase, was determined to be the primary active chitinase in the lung, and the expression of this enzyme is dependent on genetics.¹² Therefore, we sequenced the entire *CHIT1* gene to identify polymorphisms in this gene in Korean children to overcome the barrier of racial differences. These analyses revealed that *CHIT1* A442G, but not S102G and the 24-bp duplication, was associated with atopy. Blood eosinophil counts, serum IgE level, and ECP levels were higher in children with the *CHIT1* A442G CG or GG genotype than in those with the CC genotype.

To our knowledge, no other association studies of *CHIT1* A442G and other diseases, including atopy, have been reported; however, one previous study indicated that the *CHIT1* A442G polymorphism was not associated with a reduction of enzyme activity, but, on the contrary, the A442V polymorphism was associated with reduced activity in those of African ancestry.²¹ In our study, the A442V polymorphism was not detected, and only one A442G GG homozygote was identified in our Korean cohort. This locus showed allele diversity, and the allele frequencies are dependent on ethnicity.²¹

Our study revealed a new association of genotype with atopy through whole gene sequencing in Korean children. Furthermore, we found that the relative protein expression levels of chitotriosidase were higher in both cell lysate and media with the G transfection than in those with the wild type. In the future, in-depth functional studies of the risk allele G will be useful for understanding this association.

The amino acid variant S102G did not show an association with atopy in our study. Our results are consistent with previous results.^{13,22} The relationship between the S102G polymorphism and its enzyme activity is controversial.^{21,23} With regard to the 24-bp duplication, one study of a white population and another study of populations with African, European, and Asian ancestry observed the following 24-bp duplication allelic frequencies: 18%, 7%, 17%, and 56%, respectively,^{13,21} in agreement with information that alleles differ among ethnic groups. In our study, 49.8% were heterozygotes and 34.1% were homozygotes. The allelic frequency of the 24-bp duplication was 59.0%. Furthermore, the distributions were similar between the atopic and nonatopic groups, and this finding corresponds to those of the earlier study that reported that 24-bp duplication variants are not associated with asthma.^{13,16,22} The reason that the prevalence of the 24-bp duplication with or without chitotriosidase deficiency exists at such high frequency within the general population, especially in Asians including Koreans, is intriguing.²¹

Although chitinase-like proteins, such as YKL-40/BRP-39, have been suggested to associate with asthma and allergic inflammation,²⁴ the role of chitotriosidase in allergic diseases is still unclear. With regard to chitotriosidase activity, the 24-bp duplication in exon 10 has been the name of its activity in many studies because this allele generates an enzymatically inactive protein that causes chitotriosidase deficiency.²⁵ Therefore, many studies of chitotriosidase presume that 24-bp duplication heterozygotes or homozygotes may exhibit enzyme deficiency.^{3,13,17,21,22} However, 11.47% of individuals have chitotriosidase deficiency, whereas homozygosity for the 24-bp duplication is only found in 9.01% of individuals.²⁶ In addition, no differences in enzyme activities among genotypes were found, suggesting an imperfect genotype-phenotype correlation due to either an inaccurate biochemical assay or perhaps a compensational mechanism among the human chitinases chitotriosidase, AMCase, and YKL-40.²⁷ These chitinases have been found to be expressed and to function independently, although these enzymes may compensate each other.^{11,12,28} In addition, further study of the potential interactions between chitinases are warranted.

We hypothesize that *CHIT1* mutations become relevant only when individuals are in special situations, such as in the presence of high levels of fungi or states of immune compromise.²⁹ Two recent studies indicated that the relationship between *CHIT1* variants and asthma was modified by fungal exposure.^{16,29} One study also suggested that chitotriosidase may be associated with lung inflammation but not with asthma.¹² In our study, we found an association between *CHIT1* A442G and atopy but not with asthma. More importantly, the results in the replication stage also showed a more significant association between these polymorphisms and atopy. The community character of Jeju Island in Korea is relatively isolated, and its environment is more homogeneous than that of other places. We predict that the greater significance in the replication cohort represents the genetic inheritance pattern of this population. In addition, the 24-bp duplication would tend to be a risk polymorphism in the Jeju cohort. As mentioned, chitotriosidase has been found to be associated with lung inflammation, including allergic inflammation.^{10,11,30} Therefore, we infer that *CHIT1* mutations increase the risk of allergic inflammation, thereby facilitating sensitization, although we did not find that *CHIT1* specifically contributes to the development of asthma.

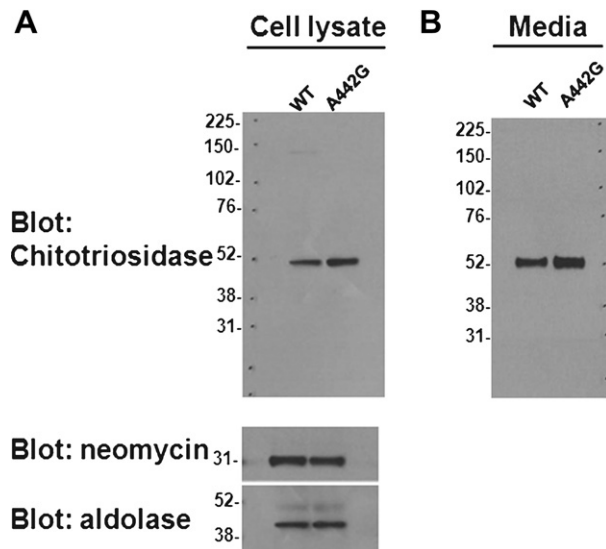
In summary, we sequenced the entire *CHIT1* gene to identify polymorphisms and then analyzed the genetic variations of the polymorphisms S102G, 24-bp duplication, and A442G in Korean children. We found that *CHIT1* A442G, but not S102G or the 24-bp duplication, was associated with atopy frequencies and atopic biomarkers, including blood eosinophils, serum IgE, and ECP. The chitotriosidase expression levels with the G transfection were significantly higher compared with the wild type. In conclusion, these results suggest that *CHIT1* genetic variation contributes to the risk of atopy in Korean children.

Supplementary Data

Supplementary data related to this article can be found online at <http://dx.doi.org/10.1016/j.anai.2013.03.009>.

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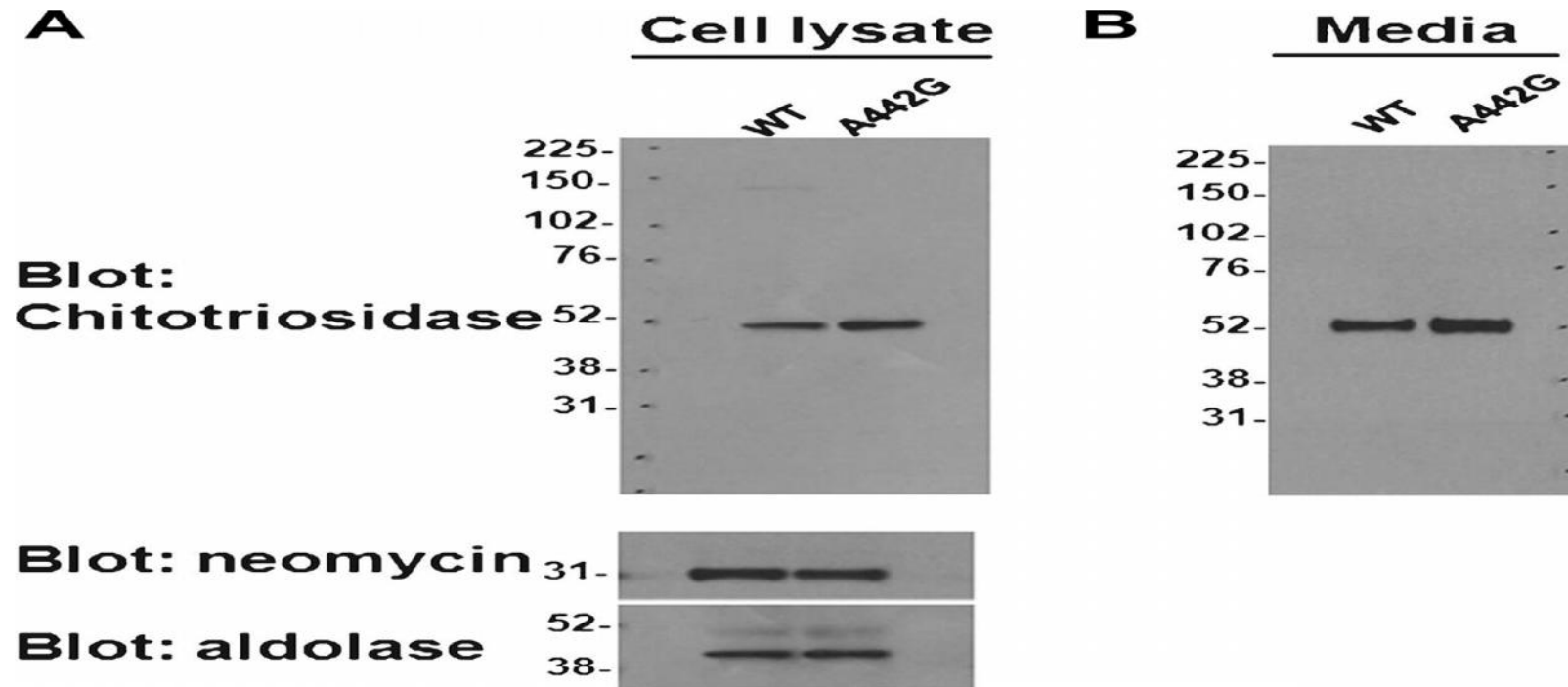
eFigure 1. Protein expression of *CHIT1* wild type (WT) and A442G variant. HEK293 cells were transfected with *CHIT1* WT and A442G clones, respectively. Total cell lysates were loaded and blotted with chitotriosidase antibody (a). Secreted chitotriosidase in media also were measured by Western blot (b).

eTable 1

Baseline characteristics of the study participants in the replication cohort

Characteristic	Atopic	Nonatopic	<i>P</i> value ^a
No. (%) of patients	202 (43.1)	267 (56.9)	
Age, mean (SD), y	10.12 (1.30)	10.17 (1.15)	.69
Male sex, No. (%)	99 (45.0)	121 (55.0)	.24
Total serum IgE levels, mean (SEM), IU/L	508.48 (32.24)	38.31 (1.88)	<.001

^a χ^2 test or *t* test was used as appropriate.



eFigure?1 Protein expression of *CHIT1* wild type (WT) and A442G variant. HEK293 cells were transfected with *CHIT1* WT and A442G clones, respectively. Total cell lysates were loaded and blotted with chitotrios...

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