Associations of a novel *IL4RA* polymorphism, Ala57Thr, in Greenlander Inuit

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Background: A novel *IL4RA* polymorphism, Ala57Thr, was identified in Greenlander Inuit.
Objective: We sought to determine whether the novel Thr57 allele is population specific and to assess the associations of Ala57Thr and Ile50Val with atopy in 2 Inuit populations.
Methods: Ala57Thr and Ile50Val were genotyped in 651 Inuit living in Denmark, 1295 Inuit living in Greenland, and 1329 individuals from 7 populations from widely differing global locations. In Inuit the polymorphisms were evaluated for associations with atopy, rhinitis, asthma, and pulmonary function.
Results: Thr57 was in linkage disequilibrium with Ile50 ($D' = 1$, $r^2 = 0.13$) and was common (33%) in the Inuit but rare (<0.6%) in all other populations. In Inuit living in Denmark, the Thr57 allele (in a dose-dependent manner) and the Ile50/Thr57 haplotype were associated with lower risk of atopy ($P_{linear} = .003$ and $P = .034$, respectively), with similar trends observed for atopic rhinitis and atopic asthma. In Inuit living in Greenland, Thr57 was not associated with atopy or atopic diseases, but Ile50 was weakly associated with lower risk of atopy.
Conclusion: The novel *IL4RA* Ala57Thr was common in and population specific to Greenlander Inuit, with Thr57 associated with a lower risk of atopy in those living in Denmark. Hence a full investigation of genotype-phenotype relationships in a given population can only be achieved if each gene is screened for novel polymorphisms in that population.
Clinical implications: Clinical risk attributable to variations in a gene in an ethnic group requires that all variations of the gene are known for that group. (J Allergy Clin Immunol 2006;118:627-34.)

Key words: IL4RA *polymorphism, atopy, asthma, rhinitis, Inuit*

Atopy is characterized by increased IgE production in response to common allergens and is strongly associated with asthma and rhinitis. The identification of genetic factors involved in the development of atopy and allergic diseases is complicated not only by its interaction with environmental exposures but also by causative heterogeneity, as well as the probability of low and age-related penetrance of multiple interacting genes.[1,2] Genome-wide screens and linkage and association studies have implicated several chromosomal regions and potential candidate genes that might be involved in the pathogenesis of atopy,[3-8] one of which is the IL-4 receptor α gene *(IL4RA)*.[4,9]

The IL-4 receptor is composed of 2 subunits: (1) the 140-kd high-affinity α chain shared by the IL-13 receptor, with both mediating many similar functions, including mediating isotype switching to IgE synthesis, and (2) the common γ chain, which is shared by several IL receptors[10] and modifies signaling of the α chain.[11]

The *IL4RA* gene is therefore an ideal candidate for atopy susceptibility because of its crucial role in allergic inflammation through its function in IL-4 and IL-13

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Abbreviations used
DRS: Dose-response slope
GM: Geometric mean
OR: Odds ratio
SNP: Single nucleotide polymorphism

signaling, as well as its location on chromosome 16p12.1-p11.2, a region previously linked to atopy and enhanced IgE responsiveness.[3-5] Many of the single nucleotide polymorphisms (SNPs) identified in the coding region of the gene have been associated with asthma and related atopic phenotypes,[12-15] either individually or in combination. The Ile50Val polymorphism, located in the extracellular domain of the receptor (amino acids numbered from the beginning of the mature protein), has been studied extensively and has been associated with IgE levels and atopic asthma,[13,16] although not all associations have been replicated in other populations.[17-19] In haplotype studies Ile50Val, together with Gln551Arg and Ser478-Pro, has been associated with atopic asthma[20,21] and total IgE levels and asthma,[22] respectively. Ile50Val has also been shown to alter receptor function in cellular assays, either in isolation[13] or in combination with Gln551Arg.[20] A study of South Dakota Hutterites and several outbred populations found that alleles, or haplotypes, showing the strongest associations differed significantly in ethnically diverse populations.[9] These data suggested either the existence of yet to be identified disease-susceptibility mutations outside the *IL4RA* gene coding region or that different risk-conferring haplotypes were population specific.

During confirmatory sequencing of the *IL4RA* region encompassing Ile50Val, we identified a novel SNP, Ala57Thr, that changes the amino acid sequence of the extracellular domain of the receptor. The SNP is a G→A substitution 21 bases downstream from Ile50Val and was found in high frequency in Greenlander Inuit populations. Given the proximity of this SNP to Ile50Val and the central role played by *IL4RA* in atopy and asthma, we hypothesized that this variant would be associated with atopy and its related diseases. This article describes the results of an investigation on the association of Ala57Thr and Ile50Val, analyzed in isolation and in combination, with atopy and other atopic diseases in 2 Inuit populations living in either Denmark or Greenland. Cohorts from diverse ethnic and geographic backgrounds were also genotyped for Ala57Thr to assess whether this SNP was population specific.

METHODS

Study populations

Inuit from Greenland and Denmark. The Inuit are indigenous to Greenland and make up approximately 90% of Greenland's population of 56,000, and an additional 8000 Inuit live in Denmark. In this study Inuit ethnicity was defined by self-identification or by having at least one Inuit parent. Between 1997 and 2001, data were collected from adult Inuit living in Greenland and Denmark, as previously described.[23,24] Briefly, Inuit in Greenland were recruited from Nuuk (the capital city), Qasigiannguit (a town), and 4 remote settlements in Uummannaq, whereas in Denmark first-generation Greenland Inuit immigrants aged between 18 and 60 years were identified from the civil registry, randomly selected, and sent a health questionnaire. In total 3327 Inuit from Greenland and Denmark were invited to participate, with the exclusion of subjects with chronic obstructive pulmonary diseases. The participation rate was 54% in Denmark and 66% in Greenland.[23] All participants were given oral and written information and provided written consent before enrollment. This study was approved by the appropriate local ethics committees in Denmark and Greenland and Princess Margaret Hospital in Perth, Australia.

Seven other populations. To determine whether the *IL4RA* Ala57Thr was population specific, cohorts from diverse ethnic and geographic backgrounds recruited for other studies were genotyped. Unless otherwise stated, they were selected without regard to their level of health or disease status and included (1) 386 Danes recruited in a prospective study of asthma and allergy in Copenhagen, Denmark[25]; (2) 91 Italians from the National Blood Bank of Italy in Genoa; (3) 117 Warao Amerindians from a community living in the Orinoco River Delta, Venezuela[26]; (4) 160 Coche Islanders of mixed ancestry (European, Amerindian, and African) from the island off the coast of Venezuela[27]; (5) 159 Ethiopians from a case-control study of asthma in Jimma, Ethiopia[28]; (6) 237 indigenous Australians from an isolated coastal community in the Kimberley region northwest of Western Australia[29]; and (7) 179 Australian children of European ancestry recruited as a birth cohort from Perth, Western Australia.[30] Approval for population polymorphism studies were obtained from each of the local ethics committees and the Ethics Committee of Princess Margaret Hospital for Children.

Phenotypic data (Inuit only)

All subjects were examined at local clinics or the university hospital (Denmark) and completed a general health questionnaire, focusing on features of atopy and respiratory symptoms. In Denmark study subjects were given the choice of either Greenlandic or Danish version of the questionnaire, whereas in Greenland, only the Greenlandic version was used.

Atopy. Subjects were considered atopic if skin test reactivity showed a mean wheal size of at least 3 mm in diameter for at least one of the 10 aeroallergens that are common and pertinent to both Greenland and Denmark. These clinically used allergens, tested in duplicate according to the European Academy of Allergology and Clinical Immunology guidelines,[31] were birch, timothy grass, mugwort, horse, dog, cat, *Dermatophagoides pteronyssinus*, and *Dermatophagoides farinae*, and 2 molds, *Alternaria alternata* and *Cladosporium herbarum*.

Rhinitis, asthma, and lung function. A general health questionnaire adapted from the International Study of Asthma and Allergy in Children[32] was designed with particular interest in respiratory symptoms. The question, ''When not having a cold, have you ever experienced having itchy eyes, sneezing, a runny nose, a blocked nose, or a diminished sense of smell?,'' was used to define rhinitis. Questions on asthma-like symptoms, such as wheezing, cough on exertion, and the use of asthma medications were used, together with self-reported asthma and physician-diagnosed asthma, with only the latter being used to define asthma. Each of the groups with asthma and rhinitis were stratified into atopic or nonatopic subgroups based on the results on the skin tests. Lung function measurements of FEV_1 and forced vital capacity, as well as bronchial hyperresponsiveness to histamine

TABLE I. Demographic characteristics of the Inuit study populations in Greenland and Denmark

	Greenland	Denmark	*P* value
Age (y)	43.5	43.4	.871
Male sex, n (%)	570 (44)	178 (27)	<.0001
Ethnicity, n (%)			
<75% Inuit heritage	137 (12)	303 (47)	<.0001
Full Inuit heritage	1052 (88)	338 (53)	
Smoking, n (%)			
Nonsmokers	229 (18)	138 (21)	.064
Previous smokers	213 (16)	119 (18)	
Current smokers	851 (66)	394 (61)	
Atopy, n (%)	46 (13)	124 (24)	<.0001
Asthma, n (%)	97 (8)	60 (9)	.238
Rhinitis, n (%)	429 (35)	395 (61)	<.0001
Allergens*	(Nuuk, Uummannaq)		
Birch pollen (total)	9-80, —	3103-9060	
Grass pollen (total)	56-103, —	1386-1723	
Cat in home	3.1, 1.7	18.6	
Dog in home	11.6, 0.9	18.6	
Pet in childhood	44.7, 12.1	53.9	
House dust mites	15.0, 3.9	72.0	
FEV_1, % predicted (SD)	98 (17)	100 (16)	.002
FEV_1/FVC, % (SD)	79.7 (7.6)	80.5 (6.3)	.023
Log DRS (SD)	0.57 (0.20)	0.53 (0.13)	.016

Total numbers for each phenotype might not add up to 1295 in Greenland and 651 in Denmark because of missing phenotypic data.
FVC, Forced vital capacity.
*In Greenland, data were obtained from Nuuk and Uummannaq.[38]

measured as the dose-response slope (DRS), were assessed as previously described.[33]

Genotyping analysis: IL4RA Ala57Thr. Of the 2046 Inuit recruited, DNA was available for 1946. Subjects were genotyped by means of restriction fragment length polymorphism assay after PCR amplification by using the primers 5′ GCA AGA GAG GCA ACC CTA 3′ (forward) and 5′ GTT ATC CGC ACT GAC CAC G 3′ (reverse). After amplification, amplicon was digested with BstU I (New England Biolabs Inc, Beverly, Mass), which digests the PCR product only when the Ala allele is present. Digested amplicons were subsequently fractioned on 2% agarose gel.

Genotyping analysis: IL4RA Ile50Val. Genotyping was performed by using the method developed by Oefner and Underhill[34] on an automated dHPLC (Varian Helix-System; Varian, Walnut Creek, Calif). A new reverse primer (5′ GCC TCC GTT GTT CTC AG 3′) was designed to avoid amplifying the SNP at position 57. PCR amplicons were then denatured at 95°C for 4 minutes and slowly cooled at 5°C intervals to 60°C, holding for 4 minutes at each temperature. Five microliters of each sample was injected into a preheated ion-paired reversed-phase column (Varian Helix Analysis Column, 3.0 × 55 mm) and eluted from the column by using a linear acetonitrile gradient at a constant flow rate of 0.45 mL/min. The optimal oven temperature in resolving the 3 genotypes was 65°C.

The genotyping results for both SNPs were confirmed by sequencing a random subset of samples (4.1% in the 2 Inuit populations and 14.8% in the non-Inuit populations) with ABI Prism BigDye Terminator Kit v3.1 (Applied Biosystems, Foster City, Calif). Although the haplotype for individuals homozygous for either SNP can be accurately determined intuitively from genotyping data, this is not possible for those heterozygous for both Ile50Val and Ala57Thr. Hence haplotypes for the Ile50Val and Ala57Thr SNPs were reconstructed by using the Bayesian statistical based program PHASE, with the phase certainty threshold set as the default (90%).[35]

TABLE II. Genotype and haplotype frequencies for the *IL4RA* SNPs in Inuit living in Greenland and Denmark

IL4RA	Greenland, n (%)	Denmark, n (%)	*P* value
Ile50Val			
Ile/Ile	878 (67.8)	333 (51.2)	<.0001
Ile/Val	359 (27.7)	271 (41.6)	
Val/Val	58 (4.5)	47 (7.2)	
Ala57Thr			
Ala/Ala	542 (41.9)	349 (53.6)	<.0001
Ala/Thr	588 (45.4)	259 (39.8)	
Thr/Thr	165 (12.7)	43 (6.6)	
Ile50Val/Ala57Thr			
Ile50/Thr57	918 (35.4)	345 (26.5)	<.0001
Ile50/Ala57	1197 (46.2)	592 (45.5)	
Val50/Ala57	475 (18.3)	365 (28.0)	

Statistical analysis

Hardy-Weinberg equilibrium for each of the SNPs was calculated by means of χ^2 goodness-of-fit tests. Linkage disequilibrium between the 2 loci was assessed by calculating the Lewontin D′ value,[36] as well as the r^2 value. All association analyses were done with logistic or linear regression for binary or quantitative phenotypes, respectively. Associations with Ile50Val and Ala57Thr were tested by using a codominant genetic model unless sample size was less than 5, whereby genotype groups for each SNP were combined, and a Val50- or Thr57-dominant model was used. When using the former model, the *P* value for linear (allele dose-dependent) trend was expressed as P_{linear}. Regression models were corrected for age, sex, ethnicity (as defined by grandparental ethnicity),[23] place of recruitment (in Greenland), current and passive smoking (for rhinitis and lung function parameters), and height (for lung function parameters). Similar to a standard method used for allele-based analysis,[37] the sample size was doubled to allow for the 2 haplotypes within each individual. This doubling of sample size was then corrected for by using robust variance estimates to allow for the duplication of the other within-person covariates in the same manner as previously described for use with twin data.[38] Multiple comparisons were not adjusted for because the analyses were based on an *a priori* hypothesis but were dealt with by describing all statistical analyses carried out.[39,40] All calculations were performed with the SPSS version 11.0 (SPSS Inc, Chicago, Ill) statistical package, except for the robust estimates, which were estimated with Stata software (StataCorp, College Station, Tex).

RESULTS

Population characteristics and genotype, allele, and haplotype frequencies

The demographic characteristics of the 2 populations (1295 Inuit living in Greenland and 651 in Denmark) are summarized in Table I. There were fewer men and fewer Inuit reporting full Inuit ancestry among the study cohort in Denmark compared with that in Greenland. Although lung function and prevalence of atopy and rhinitis were significantly different between the populations, smoking

TABLE III. ORs (95% CIs) for association analyses between phenotypes and the *IL4RA* genotypes and haplotypes in Denmark and Greenland

	IL4RA 50				
	Ile/Ile		Ile/Val		Val/Val
	n/N*	OR (95% CI)	n/N	OR (95% CI)	n/N
Denmark					
Atopy	59/271	1.14 (0.49-2.67)	57/211	1.45 (0.62-3.40)	8/37
Rhinitis	167/296	**0.35 (0.16-0.77)**	152/240	**0.45 (0.21-0.99)**	35/44
Asthma	22/296	0.44 (0.17-1.14)	19/240	0.46 (0.18-1.18)	7/44
Greenland					
Atopy	27/219	**0.27 (0.08-0.89)**	12/90	0.33 (0.09-1.16)	5/20
Rhinitis	261/760	1.34 (0.69-2.61)	113/309	1.44 (0.72-2.86)	13/47
Asthma	55/778		27/317		2/48†

Regression analyses were carried out with either Val50/Val50, Ala57/Ala57, or Val50/Ala57 as baseline and correcting for age, sex, ethnicity, place of recruitment (in Greenland) for atopy, and current and passive smoking status for rhinitis and asthma. Significant ($P < .05$) ORs and 95% CIs are presented in bold.
*n subjects with a particular phenotype out of N with that genotype; N might differ from Tables I and II because of missing data for confounders.
†Phenotype sample size was less than 5.

status differed only slightly. Exposure to allergens also differed between Greenland and Denmark (Table I).

The genotype and allele frequencies for both were significantly different between the 2 Inuit populations ($P < .0001$, Table II). The allele frequencies were 0.82 and 0.72 for Ile50 and 0.35 and 0.26 for Thr57 in Greenland and Denmark, respectively. All genotype frequencies were in Hardy-Weinberg equilibrium, except for Ile50Val in Greenland ($P = .007$), suggesting that in the latter the possibility of nonrandom mating might have influenced the distribution of the genotypes to some extent. The 2 SNPs are in linkage in both Greenland ($D' = 1.0$, $r^2 = 0.12$) and Denmark ($D' = 1.0$, $r^2 = 0.14$), with only 3 haplotypes present (Table II). The Val50/Thr57 haplotype is absent from these populations, indicating that the new Thr57 has occurred in linkage with the Ile50 allele. The frequencies for the haplotypes from both populations differed significantly ($P < .0001$, Table II).

The Thr57 allele was found in very low frequencies (<0.6%), with no homozygotes in all 7 non-Inuit populations. Only 4 Ala57/Thr57 heterozygotes were found among the Danes, 1 among the indigenous Australians, and 1 among the European Australians, with none in the Italians, Warao Indians, Coche Islanders, and Ethiopians.

Association studies

All association analyses were done separately for the 2 Inuit populations because the prevalences of the phenotypes were significantly different. Furthermore, environmental exposures, such as climate, pollen, pet, and house dust mite exposures, differed for the 2 populations[41] (Table I). All regression analyses for the Ile50Val and Thr57Ala SNPs were done by using Val50/Val50 and Ala57/Ala57 as baseline genotypes, respectively. The Val50/Ala57 haplotype was used as baseline for all haplotype analyses.

Denmark

Atopy. The *IL4RA* Thr57 allele was associated with a protective dose effect against atopy ($P_{linear} = .003$), and consistent with this, the only haplotype associated with protection against atopy was Ile50/Thr57 (odds ratio [OR] = 0.63, $P = .034$; Table III and Fig 1). There was a trend for Thr57 homozygotes to be associated with protection against atopy (OR = 0.42, $P = .095$), and similarly, individuals with the Ala57/Thr57 genotype were also protected (OR = 0.52, $P = .005$). Ile50Val was not associated with atopy (Table III and Fig 1). Severity of atopy (log total wheal sizes and log number of positive skin test reactivity, excluding nonatopic subjects) was not associated with either of the *IL4RA* polymorphisms (data not shown).

To assess the effect of the Thr57 allele independent of the Ile50Val, the latter genotype was added as a confounder in the regression analysis. The association with atopy remained (Thr57/Thr57: OR = 0.416, $P = .098$; Thr57/Ala57: OR = 0.49, $P = .003$; $P_{linear} = .003$), suggesting that the effect found with Ala57Thr was not due to its linkage with Ile50Val.

Rhinitis. Ile50Val, but not Ala57Thr, was associated with rhinitis (Table III). Individuals with one Ile50 allele had lower odds of having rhinitis (OR = 0.45, $P = .049$), with those possessing 2 alleles having the smallest risk (OR = 0.35, $P = .009$). Both haplotypes with Ile50 were associated with lower risk compared with the haplotype without Ile50 (Ile50/Thr57: OR = 0.68, $P = .015$; Ile50/Ala57: OR = 0.71, $P = .019$).

Because there was a large number of individuals with rhinitis who were not atopic (221/316 with data for atopy, rhinitis, and genotyping), further analysis was carried out to determine whether the association observed between rhinitis and Ile50Val was related to atopic status. Compared with those without rhinitis, the association with Ile50Val remained only with the nonatopic group (Ile50/Ile50: OR = 0.31, $P = .008$; Ile50/Val50: OR = 0.41, $P = .046$) and was dose dependent ($P_{linear} = .01$). Atopic rhinitis, on the other hand, was associated with Ala57Thr. Although sample size was small (n = 3), individuals with the Thr57/Thr57 genotype appeared to be less likely to have atopic rhinitis compared with those lacking the Thr57 allele. Additionally, possessing at least one Thr57

TABLE III. (continued)

IL4RA 57					*IL4RA* 50/57 haplotype				
Thr/Thr		Thr/Ala		Ala/Ala	Ile/Thr		Ile/Ala		Val/Ala
n/N	OR (95% CI)	n/N	OR (95% CI)	n/N	n/N	OR (95% CI)	n/N	OR (95% CI)	n/N
5/35	0.42 (0.15-1.16)	37/205	**0.52 (0.33-0.82)**	82/279	47/275	**0.63 (0.42-0.95)**	128/478	1.11 (0.81-1.54)	73/285
15/34	0.52 (0.24-1.09)	144/235	0.96 (0.67-1.38)	195/311	174/303	**0.68 (0.50-0.93)**	212/429	**0.71 (0.53-0.94)**	222/328
2/34†		16/235	0.70 (0.37-1.35)	30/311	20/303	0.66 (0.36-1.20)	43/529	0.80 (0.50-1.28)	33/328
4/41†		18/152	0.67 (0.33-1.36)	22/136	26/234	0.54 (0.27-1.10)	40/294	0.67 (0.35-1.28)	22/130
52/141	1.03 (0.69-1.54)	165/504	0.85 (0.65-1.11)	170/471	269/786	1.00 (0.77-1.31)	366/1043	1.05 (0.82-1.35)	199/463
8/143	0.61 (0.27-1.38)	37/520	0.87 (0.53-1.40)	39/480	53/806	0.81 (0.51-1.28)	84/1067	0.98 (0.63-1.51)	31/413

allele conferred protection against atopic rhinitis (OR = 0.53, $P = .029$). The only haplotype with the Thr57 allele was associated with protection against atopic rhinitis (OR = 0.52, $P = .012$), whereas the haplotypes with Ile50 protected against nonatopic rhinitis (Ile50/Thr57: OR = 0.69, $P = .049$; Ile50/Ala57: OR = 0.62, $P = .009$; see Table E1 in the Online Repository at www.jacionline.org).

Asthma and lung function parameters. No associations were found between asthma and the 2 *IL4RA* SNPs, either individually (in a codominant or Val50-dominant model) or in combination (Table III). However, when the asthmatic group was stratified by atopic status and compared with those without asthma, only Ala57Thr appeared to be associated with atopic asthma. None of the 28 Inuit with available phenotypic data who had the Thr57/Thr57 genotype had atopic asthma, and although the sample size was small (n = 3), it appeared that those who were heterozygous for the Thr57Ala SNP were less likely to have atopic asthma (see Table E1 in the Online Repository at www.jacionline.org). Although haplotype analyses were not carried out for Ile50Thr57 because of small phenotype sample sizes (n < 5), individuals with this haplotype appeared to be protected against atopic asthma. No associations were found with the nonatopic subgroup.

FEV_1 percent predicted values and FEV_1/forced vital capacity ratios were not associated with the *IL4RA* polymorphisms. Subjects with the Ile50 allele had lower DRSs compared with those without (geometric mean [GM] DRS [percentage decrease in FEV_1/μM histamine): Ile50/Ile50 GM = 0.38, $P = .04$; Ile50/Val50 GM = 0.40, $P = .036$; Val50/Val50 GM = 0.89). No association was found between the Thr57Ala polymorphism and DRS (data not shown).

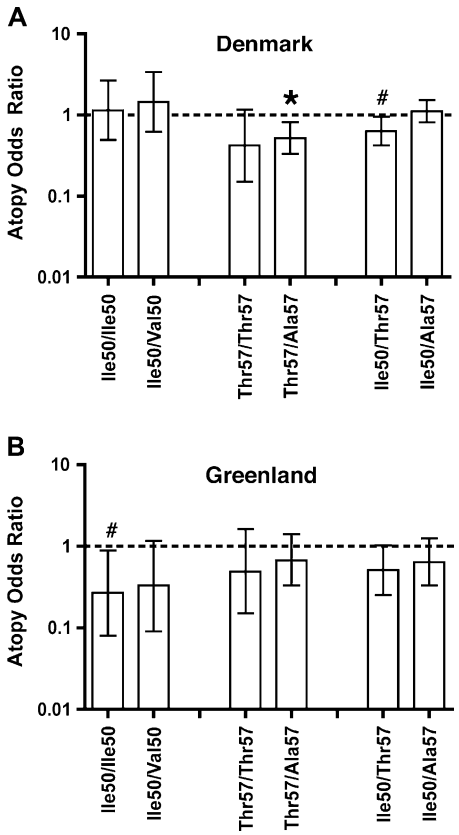

FIG 1. ORs for atopy associated with *IL4RA* genotypes and haplotypes compared with Val50/Val50, Ala57/Ala57, or Val50/Ala57 in Denmark **(A)** and in Greenland **(B)**. *Error bars* represent 95% CIs. #$P < .01$. *$P < .05$.

Greenland

Atopy. There were only 44 atopic individuals in the cohort living in Greenland. Subjects homozygous for the Ile50 allele had the lowest risk of atopy (OR = 0.27, $P = .032$; Table I). Those heterozygous for the SNP also tended to have a lower risk of atopy, although the effect was not quite significant (OR = 0.33, $P = .084$). However, there were no associations between Ala57Thr (in a Thr57-dominant model) or the haplotypes and atopy, even though a trend was observed with the Ile50/Thr57 haplotype (OR = 0.54, $P = .092$). Severity of atopy

was not associated with either of the *IL4RA* polymorphisms (data not shown).

Rhinitis. There was no association between the *IL4RA* SNPs, either individually or in combination, and rhinitis, despite the sufficiently large number of subjects with the condition (n = 387, Table III). This lack of association was also observed for both atopic and nonatopic rhinitis.

Asthma and lung function parameters. Neither the *IL4RA* polymorphisms nor haplotypes were associated with asthma (Table III) or any of the lung function parameters (data not shown).

DISCUSSION

This study reported a novel *IL4RA* polymorphism that was present in Inuit but rare or absent in all other populations. The Thr57 allele of this polymorphism was associated with a lower risk of atopy in the Inuit in Denmark. Important implications of these findings are as follows: (1) when investigating for genotype-phenotype relationships in a new population, each gene needs to be screened for novel polymorphisms that might be unique to that population, and (2) assessing the same population group living in 2 distinctly different environments facilitates analysis of the influence of environmental factors independent of genetics.

The novel Thr57 allele, which appeared to be a recent mutation that had occurred in linkage with the Ile50 allele, was highly specific to the Greenlander Inuit (frequency of 32.5%) but was almost nonexistent in several other global populations (frequency of <0.6%). Although there have been many reports of ethnic- or population-specific polymorphisms, most of these alleles were found in low frequency,[42,43] and those in high frequency (>25%) were mainly confined to African and African American populations.[42] Moreover, most of these studies, which aimed to identify polymorphisms in a large number of genes, have been limited either by the small number of ethnic cohorts or a paucity of individuals studied in each population.[42,44,45] This study differed from previous investigations in that the cohorts were from general, as well as isolated, populations and hence lacked the genetic admixture found in most studied populations. Furthermore, all the populations had sufficient sample size to provide a 95% chance of successfully detecting the SNP if the minimal allele frequency in any was 1%.[46]

Possible explanations for the population differences in the Thr57 allele frequencies include the differential period at which the mutation occurred, founder effect and random drift in a smallish isolated population, or natural selection favoring the Thr57 allele in the Inuit population as an adaptive strategy to deal with the arctic environment. Although founder effect cannot be ruled out, the possibility also remains that natural selection might be responsible. The natural selection explanation is consistent with the postulate that the adaptation of the human immune system to the environment over many millennia might play a crucial role in current susceptibility to atopy and asthma.[47] Long-term exposure to parasites in tropical populations might have selected for an augmented immune response, as reflected in their high prevalence of proinflammatory alleles.[48] On the contrary, populations with long-term residence in temperate or arctic regions do not require an upregulated proinflammatory immune system, which might result in deleterious overreactions to other normally harmless environmental agents. Hence having a less proinflammatory immune system would provide a survival advantage to the Inuit, who have lived in an arctic environment for at least 19,000 years.[49] The *IL4RA* Thr57 allele could have been selected for because it is associated with a lower risk of atopy.

This study found an association between the *IL4RA* Thr57 allele and a reduced risk of development of atopy in the Inuit living in Denmark. The protective association seemed to extend to atopic rhinitis and atopic asthma in Denmark but not to atopic severity. Evidence supporting this association includes the following: (1) the *IL4RA* gene is located in a region that has been previously linked to atopy,[3-5] and (2) the substitution of a polar threonine for the small nonpolar alanine within the important extracellular domain of the receptor could result in a functional change. Alternately, the association observed in this study could be the result of linkage disequilibrium between Ala57Thr and another putative disease-causing mutation or epistatic effects caused by interaction with other SNPs in *IL4RA* or other genes. Because data on linkage disequilibrium patterns in the Inuit would be ideal to resolve the former, sequencing the gene and genotyping other known SNPs within the gene and adjacent genes are now planned for future studies. Further studies are also underway to elucidate the effect of this SNP on atopy and receptor function and to assess multiloci interactions.

As with asthma, atopy is a disease state resulting from gene-environment interactions. Because the Inuit populations studied were of a similar genetic background (with corrections for ''degree of ethnicity'' included in all regression models), the influence of the environment per se could be assessed. The effect of the Thr57 allele was evident in the first-generation Inuit migrants in Denmark but not in the Inuit in Greenland, although similar trends and ORs were observed. Although the possibility exists that there was insufficient power to detect any associations in the latter because of small sample size, it is also probable that it is due to the differing levels of allergens in the 2 locations. With birch and grass pollen counts negligible in Qasigiannguit and Uummannaq and 100 times less in Nuuk than in Denmark,[41] an allergen sensitization threshold might not have been reached in Greenland, resulting in nonpenetrance. This could explain why different population studies are inconsistent in reported associations between specific alleles and atopic phenotypes.[13,16,18] Alternately, the inconsistent association found could be due to different linkage disequilibrium patterns in the 2 populations or gene-gene interactions.

The association of the Ile50Val SNP with atopy has been well documented,[13,16] but not all studies have found a significant relationship.[17,18] In the current study the

association was only observed in Greenland, with the Ile50 allele associated with reduced atopy, as opposed to increased atopy in the Japanese,[13,16] but this latter finding needs to be replicated in a larger cohort, considering the small sample size of 44 atopic individuals. Nevertheless, the results do suggest that the Ile50 allele would be associated with reduced inflammation. This might explain the diminished airway responsiveness, as assessed on the basis of DRS, in individuals with the allele in Denmark, even though a significant association with atopy was not observed.

The association between Ile50Val and nonatopic rhinitis is a novel finding. The main trigger of such ''nonatopic'' (as defined by skin test reactivity) rhinitis is any particulate matter in the air, including pollen, smoke, or other irritants, and allergens that do not cause an allergic skin reaction might still be nasal irritants, as in the case of pollen. Hence individuals with a less proinflammatory genetic profile might be at lower risk of nonallergic rhinitis. Furthermore, a study on nasal turbinates obtained from patients with nonallergic rhinitis[50] found the Val50 variant of the receptor, in combination with Arg551, exhibited enhanced sensitivity to IL-4,[20] a major stimulus for the production of the potent eosinophil attractant eotaxin 2/CCL24.

In this study multiple comparisons were performed to study the associations between often correlated phenotypes and genotypes that are in linkage disequilibrium. We have not corrected for such multiple testing (1) because it can be overly conservative, especially correcting with the commonly used Bonferroni method, and (2) because the analyses were based on an *a priori* hypothesis, we have chosen to describe all statistical analyses carried out and discuss the possible interpretations of each result.[39,40] Furthermore, the associations found in this study between the *IL4RA* SNPs and atopy are biologically plausible and are supported by existing literature: (1) the *IL4RA* gene is involved in expression of the phenotype, and (2) previous studies on polymorphisms in the same gene have shown similar associations.[12-15]

In conclusion, we have found a novel polymorphism, *IL4RA* Ala57Thr, in a gene in a key immunologic pathway. The polymorphism was common in an isolated arctic population but almost nonexistent in populations from all other locations tested. Evidence suggests that the polymorphism might be associated with a reduction in the tendency toward atopy. The population-specific nature of the SNP highlights the need for comprehensive screening for unique sequence variants within candidate genes in multiple populations, and the high frequency of the Thr57 allele in the Inuit suggested the possibly that it became fixed through natural selection. Additional data, such as serum IgE levels and further gene and protein functional studies, are under way to elucidate the mechanisms underlying the genetic associations.

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