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Original Article

Association analyses of eQTLs of the *TYRO3* gene and allergic diseases in Japanese populations

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eQTL, expression quantitative trait locus; OR, odds ratio; GWAS, genome-wide association study; PARF, population attributable risk fraction; MAF, minor allele frequency; LD, linkage disequilibrium

ABSTRACT

Background: *TYRO3* is a member of the TAM (*TYRO3*, *AXL*, *MERTK*) receptor tyrosine kinase family and functions to limit type 2 immune responses implicated in allergic sensitization. Recent studies have shown that multiple intronic variants of *TYRO3* were associated with asthma, implying that genetic variation could contribute to errant immune activation. We therefore hypothesized that expression quantitative trait loci (eQTLs) of the *TYRO3* gene influence the development of allergic diseases (including asthma and allergic rhinitis) in Japanese populations.

Methods: We performed a candidate gene case–control association study of 8 eQTLs of *TYRO3* on atopy, asthma, and allergic rhinitis using 1168 unrelated Japanese adults who had GWAS genotyping. We then examined the genetic impact of rs2297377 (*TYRO3*) on atopy and allergic rhinitis in 2 other independent Japanese populations.

Results: A meta-analysis of 3 Japanese populations (a total of 2403 Japanese adults) revealed that rs2297377 was associated with atopy and allergic rhinitis (OR = 1.29 and 1.31; P = 0.00041 and 0.0010, respectively). The risk allele at rs2297377 correlated with decreased expression of *TYRO3* mRNA. The gene–gene interaction between *HLA-DPB1* and *TYRO3* was not significant with regard to sensitization. The estimated proportion of atopy and allergic rhinitis cases attributable to the risk genotype was 14% and 16%, respectively.

Conclusions: Our study identified *TYRO3* as an important susceptibility gene to atopy and allergic rhinitis in Japanese.

Introduction

Type 2 immune responses, characterized by the differentiation of CD4+ T helper type 2 (Th2) cells and the production of type 2 cytokines such as interleukin-4 (IL-4), IL-5, IL-9 and IL-13, play a critical role in the pathobiology of allergic diseases such as asthma and allergic rhinitis. Recently, genome-wide association studies have revealed a link between the *TYRO3* gene and asthma in African American and Latino patients.[1] TYRO3 is a member of the TAM (TYRO3, AXL, and MERTK) family of receptor tyrosine kinases expressed on dendritic cells and its activation functions as a negative regulator of type 2 immune responses that mediate both immunoactivation and immunopathology.[1] In this study, to examine the genetic impact of *TYRO3* on the development of atopy, asthma and allergic rhinitis in Japanese individuals, we performed a candidate gene case–control association study on 2403 samples from 3 independent Japanese populations. We also assessed the contribution of *TYRO3* in estimating the population-attributable risk for the general Japanese population.

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Methods

Ethics statement

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

Participant pools

We studied 3 Japanese adult populations. All participants were unrelated Japanese adults, recruited within Ibaraki Prefecture, Japan from June 2008 to March 2016. The first population (Tsukuba Cohort 1) consisted of 201 patients with asthma and 967 healthy adults. In this population, all participants had genome-wide SNP typing.[2] The second population (Tsukuba Cohort 2) consisted of 190 patients with asthma and 514 healthy adults.[3,4] In these 2 populations, asthmatic patients were recruited from the Tsukuba University Hospital and its affiliated hospitals. Healthy adults without respiratory diseases were recruited from persons who visited the Tsukuba Medical Center for annual health checkups. Patients were considered asthmatic on the basis of recurrent episodes of two or more of the three symptoms (coughing, wheezing, or dyspnea) associated with demonstrable reversible airflow obstruction and/or increased airway responsiveness to methacholine.[5] Healthy adults in both populations had no pulmonary diseases such as asthma or COPD. 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. Allergic rhinitis was defined as the presence of paroxysms of one or more of three symptoms (sneezing, rhinorrhea, or nasal obstruction) with atopy.[6]

The third population consisted of 531 students (102 patients with asthma) enrolled in medicine, nursing or medical sciences at the University of Tsukuba from 2013 to 2015 (Tsukuba Student Cohort).[7] In this population, allergen-specific IgE antibody was measured only for Japanese cedar pollen and *D. farinae* by the CAP-FEIA method (Phadia AB, Uppsala, Sweden). Atopy was defined by the presence of specific IgE antibody towards either *D. farinae* or Japanese cedar and the diagnosis of allergic rhinitis was based on the presence of both atopy and nasal symptoms (sneezing, rhinorrhea, or nasal obstruction). Asthmatic patients were excluded from analyses of atopy and allergic rhinitis since atopy is secondary to asthma in some individuals and, as it is not a primary driver of the disease,[8] its presence could confound the genetic analysis results.

Genotyping

Genomic DNA was extracted from whole blood by an automated DNA extraction system (QuickGene-610L; Fujifilm, Tokyo, Japan). In Tsukuba Cohort 1, 201 patients with asthma and 967 healthy control adults underwent GWAS genotyping using Illumina HumanHap 550k v3/610-Quad BeadChips (Illumina, San Diego, CA, USA) as described previously.[2] The TaqMan allele-specific amplification method was used to define genotypes for rs2297377 in both Tsukuba Cohort 2 and the Tsukuba Student Cohort (Applied Biosystems, Foster City, CA, USA).

Statistical analysis

SNPs in and around the *TYRO3* gene were examined for associations with allergic diseases using logistic regression modeling. For rs2297377, combined results from 3 independent cohorts were analyzed by adjusting for the cohort in a logistic regression model. To quantify the effect of heterogeneity according to the population, we also calculated the I^2 heterogeneity index using PLINK ver. 1.07 (Command:–meta-analysis), which describes the percentage of total variation across populations that is due to heterogeneity rather than chance.[9] Hardy–Weinberg equilibrium was confirmed for the genotype frequencies of the 3 populations.

The population attributable risk fractions (PARF) for atopy and allergic rhinitis were examined using the following formula: $PARF = P(E)(OR-1)/[1 + P(E)(OR-1)]$, where P(E) is the probability of exposure, in this case the probability of having the risk genotype, and OR refers to the odds of having atopy and allergic rhinitis for individuals with the risk genotype as opposed to the odds of having atopy and allergic rhinitis for individuals with non-risk genotypes.

All analyses were performed in SPSS (version 24). P values of less than 0.05 were considered statistically significant. Given that there were *a priori* reasons for suspecting the *TYRO3* gene would be linked to allergic disease risk, our data were not corrected for multiple testing.

Results

Lung tissue shows eQTLs are associated with TYRO3 mRNA expression

The characteristics of Tsukuba Cohort 1, Tsukuba Cohort 2 and the Tsukuba Student Cohort are shown in Table 1. The strongest association with asthma in the previous meta-analysis of non-Japanese GWAS was found with rs1200341.[1] However, the minor allele frequency (MAF) of this SNP was found to be very rare (0.8%) in Tsukuba Cohort 1. As variation in gene expression is an important exploitative mechanism to boost statistical power and enhance interpretation in genome-wide association studies (GWASs) of underlying susceptibility to complex diseases,[10] we initially searched cis-eQTLs of *TYRO3* that reside within 1 Mb of the transcription start site on the *TYRO3* gene with the GTEx Portal database (https://gtexportal.org/home/). A total of 192 SNPs associated with lung levels of *TYRO3* mRNA were found of which 8 were selected for genetic analyses. Criteria for these analyses were strong association with *TYRO3* mRNA expression in lung tissues, P values of less than 5×10^{-10}, and a MAF of more than 10% in the HapMap-JPT (Japanese in Tokyo, Japan, Phase 3) (Table 2).

TYRO3 eQTLs are associated with allergic diseases

In Tsukuba Cohort 1, rs2297377 was associated with atopy ($p = 0.043$) and allergic rhinitis ($p = 0.019$) while rs1473781 was associated with allergic rhinitis ($p = 0.033$) (Table 2). None of the 8 chosen SNPs were associated with the presence of asthma even when the analysis was restricted to atopic asthma (Table 2). Given that rs2297377 showed the strongest significant association with atopy and allergic rhinitis, we then examined these associations in both Tsukuba Cohort 2 and the Tsukuba Student Cohort. In Tsukuba Cohort 2, rs2297377 was again associated with atopy and allergic rhinitis (OR = 1.44 and 1.46; $p = 0.0059$ and 0.016, respectively) (Table 3). In the Tsukuba Student Cohort, rs229377 was associated with atopy when the carrier of the risk allele was compared with non-carriers (OR = 2.13; $p = 0.026$). In the combined meta-analysis

Table 1
Characteristics of study population.

	Tsukuba cohort 1		Tsukuba cohort 2		Tsukuba student cohort	
	nonasthmatics	asthmatics	nonasthmatics	asthmatics	nonasthmatics	asthmatics
Number of subjects	967	201	514	190	429	102
Sex (female, %)	526 (54.4)	118 (58.7)	263 (51.2)	117 (61.9)	257 (59.9)	60 (58.8)
Age, y (range)	50.0 (27–74)	51.0 (20–72)	51.2 (22–81)	56.8 (18–84)	19.8 (18–38)	19.9 (19–29)
Age of asthma onset (range)		36.9 (0–70)		39.1 (1–82)		NA
Smoking (%)						
Pack-year						
0	607 (62.8)	164 (82.0)	309 (60.1)	122 (64.2)	NA	NA
0–10	127 (13.1)	33 (16.5)	64 (12.5)	21 (11.1)	NA	NA
>10	233 (24.1)	3 (1.5)	141 (27.4)	46 (24.2)	NA	NA
Atopy (%)	541 (55.9)	143 (71.1)	328 (63.8)	137 (72.1)	334 (77.9)	94 (92.2)
Allergic rhinitis (%)	358 (37.0)	69 (34.3)	207 (40.3)	36 (18.9)	163 (67.9)	60 (58.8)
Serum IgE (log, SD)	1.73 (0.56)	2.23 (0.62)	1.93 (0.64)	2.26 (0.60)	2.15 (0.61)	2.47 (0.63)

Information on asthma onset age and smoking was missing in 3 and 1 patients with asthma in the Tsukuba cohort 2, respectively.
Information on serum IgE was missing in 2 and 4 patients with asthma in the Tsukuba cohort 1 and 2, respectively.

Table 2
Association of eQTL SNPs of *TYRO3* with allergic diseases in Tsukuba cohort 1.

SNP	*P* value	Minor allele	Minor allele frequency		*P* value		
	eQTL		HapMap JPT	Tsukuba cohort 1 (nonasthmatics + asthmatics)	Asthma	Atopy	Allergic rhinitis
rs9944249	7.0×10^{-34}	T	0.15	0.14	0.39	0.52	0.44
rs721772	6.4×10^{-24}	A	0.44	0.39	0.92	0.89	0.51
rs2297381	1.3×10^{-23}	C	0.44	0.40	0.77	0.76	0.33
rs1200345	1.7×10^{-17}	T	0.33	0.30	0.73	0.19	0.12
rs1473781	1.9×10^{-17}	A	0.16	0.14	0.52	0.14	**0.033**
rs1200353	1.0×10^{-15}	T	0.15	0.13	0.68	0.18	0.095
rs2297380	1.8×10^{-15}	T	0.40	0.41	0.86	0.42	0.15
rs2297377	1.5×10^{-14}	A	0.31	0.32	0.38	**0.043**	**0.019**

Significant differences are highlighted in bold ($p < 0.05$).

Table 3
Results of association analysis in 3 populations and meta-analysis (rs2297377).

	Tsukuba cohort 1				Tsukuba cohort 2				Tsukuba student cohort				Meta-analysis	
	n	RAF (G)	OR (95% CI)	*P* value	n	RAF (G)	OR (95% CI)	*P* value	n	RAF (G)	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Asthmatics	201	0.66	0.91	0.38	190	0.65	0.89	0.35	102	0.65	0.94	0.70	0.91	0.19
Nonasthmatics	967	0.68	(0.72–1.13)		514	0.68	(0.70–1.14)		429	0.66	(0.68–1.30)		(0.78–1.05)	
Atopy (−)	426	0.66	**1.22**	**0.043**	186	0.62	**1.44**	**0.0059**	95	0.62	1.27	0.17	**1.29**	**0.00041**
Atopy (+)	541	0.70	(1.01–1.47)		328	0.71	(1.11–1.87)		334	0.67	(0.90–1.81)		(1.12–1.48)	
Allergic rhinitis (−)	370	0.66	**1.30**	**0.019**	152	0.63	**1.46**	**0.016**	77	0.64	1.14	0.53	**1.31**	**0.0010**
Allergic rhinitis (+)	358	0.72	(1.04–1.61)		207	0.72	(1.07–1.99)		163	0.67	(0.76–1.70)		(1.12–1.55)	

RAF, risk allele frequency.
Significant differences are highlighted in bold ($p < 0.05$).

of the 3 independent populations, the presence of the G allele was found to be associated with atopy and allergic rhinitis (OR = 1.29 and 1.31; P = 0.00041 and 0.0010, respectively) (Table 3) while the risk allele G that is associated with the presence of atopy and allergic rhinitis was significantly correlated with decreased expression of *TYRO3* mRNA in the lung. I^2 was 0 for asthma (p for Cochrane's Q statistic = 0.97), allergic sensitization (0.58) and/or allergic rhinitis (0.62), revealing that there is no clear heterogeneity according to the population for the association between rs2297377 and atopy or allergic rhinitis.

Gene–gene interaction between HLA-DPB1 and TYRO3 is not significant with regard to allergic sensitization

Dendritic cells (DCs) present peptide antigen via MHC class II to stimulate naïve T cells and effect an active response while TYRO3, interacting with Protein S on the DCs, engages a negative feedback mechanism that specifically inhibits type 2 inflammation.[11] As the significance of associations between *HLA-DPB1* genotypes and Japanese cedar sensitization was previously established,[7] we therefore examined this push–pull relationship by studying the genetic influence of *TYRO3* on the significance of associations between *HLA-DPB1* genotypes and Japanese cedar sensitization in the Tsukuba student cohort. We found that the effect of rs229377 genotype on the effects of *HLA-DPB1* alleles with regard to allergic sensitization was not significant (p > 0.1 for interaction term) (Table 4).

rs229377 population attributable risk for atopy and allergic rhinitis

In the estimation of the population attributable risk fraction (PARF) for atopy and allergic rhinitis, we used a recessive model that provided a better fit than dominant modeling as revealed by

Table 4
The interaction of rs2297377 genotype with *HLA-DPB1* allele.

rs2297377 genotype	JC sensitization (%)	*HLA-DPB1*02*					*HLA-DPB1*05*				
		Frequency (%)	Case Count (%)	Control Count (%)	*P* value	OR (95% CI)	Frequency (%)	Case Count (%)	Control Count (%)	*P* value	OR (95% CI)
Total (n = 429)	308 (71.8)	24.9	134 (21.8)	80 (33.1)	0.00082	0.57 (0.41–0.79)	39.2	253 (41.1)	83 (34.3)	0.10	1.28 (0.95–1.73)
AA (n = 42)	24 (57.1)	22.6	8 (16.7)	11 (30.6)	0.14	0.44 (0.15–1.31)	35.7	17 (35.4)	13 (36.1)	0.95	0.97 (0.39–2.43)
AG (n = 207)	155 (74.9)	26.1	74 (23.9)	34 (32.7)	0.079	0.64 (0.39–1.05)	39.6	127 (41.0)	37 (35.6)	0.35	1.24 (0.79–1.94)
GG (n = 180)	129 (71.7)	24.2	52 (20.2)	35 (34.3)	0.0077	0.50 (0.30–0.83)	37.8	109 (42.2)	33 (32.4)	0.10	1.47 (0.93–2.33)
					P for interaction = 0.42					*P* for interaction = 0.72	

the lower P value using the 2 × 2 contingency table. The proportion of atopic and allergic rhinitis cases statistically attributable to the rs229377 G allele at *TYRO3* was 14% and 16%, respectively, in our Japanese populations.

Discussion

In the current study, we have shown the importance of the *TYRO3* functional polymorphism that alters gene expression in the pathogenesis of allergic sensitization and allergic rhinitis. Our meta-analysis of 3 independent Japanese populations showed that the rs2297377 at the *TYRO3* is negatively associated with the development of allergic sensitization and allergic rhinitis. The exact mechanism for this is unclear but the evidence points to the lack of TAM family receptor tyrosine kinase TYRO3 expression in lung dendritic cells (DCs) which drives enhanced production of type 2 chemokines and an exacerbated type 2 immune response.[1] Our findings in the current study, therefore, suggest that the mutated *TYRO3* genotype that correlates with lower mRNA expression leads to an enhanced type 2 immunity which subsequently results in an increased susceptibility to allergic sensitization and allergic rhinitis.

It is of interest to note that, when the microarray analysis was conducted on mouse bone marrow–derived cultured mast cells (MCs) in the presence of IL-33 for 4 weeks, *TYRO3* was strongly upregulated.[12] It is suggested that IL-33 has some role in resolution of the exaggerated type-2 immune response by tempering further MC-dependent allergic inflammation under chronic conditions.[12] Therefore, the failure of IL-33 to downregulate MC activation through insufficient expression of a negative regulator of type 2 immune responses, TYRO3, may at least partly explain our findings of the association between rs2297377 and allergic sensitization.

The original meta-analysis of GWAS identified *TYRO3* as a susceptibility gene to asthma.[1] In our study, however, rs2297377 was not associated with asthma and this discrepancy may be partly explained by the heterogeneity of asthma. First of all, patients with asthma in the original GWAS were predominantly early-onset and atopic.[13,14] In contrast, in both Tsukuba cohorts (1 and 2), the median of asthma onset age was approximately 38 years old and the presence of atopy in patients with asthma was less frequent (approximately 72%) compared to the original study. In addition, given that the TAM family also functions as an apoptotic cell recognition receptor (controlling the duration of the immune response) and given that apoptotic cell removal is defective in non-eosinophilic asthma,[15] dysregulated activation of apoptotic cell recognition receptors may rather exaggerate inflammatory responses leading to subsequent development of asthma in certain disease subtypes. We believe that the genetic impact of *TYRO3* therefore needs to be examined in larger populations with a focus on specific asthma phenotypes.

We acknowledge several limitations of our study. The homogeneity of our study populations may limit the applicability of our results to populations of different ancestries. Lack of population diversity might also increase the size of LD blocks and thereby limit the resolution with which true regulatory sites can be identified. In addition, a false-positive result may serve as an alternate explanation for the modest association of *TYRO3* with allergic sensitization and allergic rhinitis in our study. Possible explanations for a false-positive result may include technical artifacts, population stratification, or an inappropriate statistical threshold of significance but our genotyping efficiency and accuracy argue strongly against technical artifacts. Additionally, all cohorts were comprised of Japanese adults recruited in Ibaraki Prefecture, Japan which reduces the likelihood of stratification. Ultimately, additional studies in sufficiently powered cohorts of patients with allergic diseases, together with stratification according to disease phenotypes, are required to definitively determine whether genetic variations within *TYRO3* influence the risk of developing allergic diseases. Finally, it is clear that studies attempting to accurately define population-attributable risks for a specific polymorphism in risk genes for common polygenic disorders (such as allergies) will require very large study populations.

In conclusion, our findings provide deeper insight into the genetic contribution of *TYRO3* in the development of allergic diseases in Japanese populations. Given its population risk attribution of more than 10% for atopy and allergic rhinitis, TYRO3 may be an attractive pharmacological target for treating allergic diseases such as Japanese cedar pollinosis.

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Conflict of interest

NH received lecture fees and/or research funding from AstraZeneca, Astellas Pharma, Boehringer Ingelheim, MSD, Novartis, Ono Pharmaceutical, and Pfizer. The rest of the authors have no conflict of interest.

Authors' contributions

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

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