Although the SNP in the *PYHIN1/IFI16* region did not reach genome-wide significance in the primary GWAS ($P = 3.19 \times 10^{-7}$), a recent meta-analysis of GWASs of asthma has identified a SNP in *PYHIN1* in populations of African descent [40]. PYHIN1 and IFI16 have recently emerged as sensors of microbial DNA [41], and the innate immune response relies on the ability of immune cells to detect the presence of infection through these germline-encoded pattern recognition receptors. Given that environments with a wide range of microbial exposures are associated with protection from childhood asthma and atopy in proportion to their level of exposure to bacterial and fungal microbes [42], *PYHIN1* and *IFI16* deserve further attention as candidate genes for association with asthma and atopy.

We have here tried to validate previously reported gene associations with IgE regulation. Among 32 autosomal genes, which were previously identified mainly in European populations, we found that 9 (28.1%) were replicated in a Japanese population after correction for multiple testing, indicating that some of the candidate genes for association with total serum IgE levels are effective across ethnic groups. Heterogeneity seems to exist in the genetic factors for total serum IgE levels among different ethnic groups [13,43]. For SNPs that were not replicated in our study, causal SNPs for IgE regulation or SNPs tightly in LD with the causal SNPs may not exist in the regions analyzed in the Japanese population. Alternatively, our study sample size may have not have provided a significant power to detect the associations due to low minor allele frequencies of the true causal SNPs.

In terms of the limitations of this study, because we chose only 1 SNP for each region to replicate the original findings in the discovery cohort, we cannot exclude the possibility that we have missed true functional genetic variants in the replication cohorts. In addition, we observed many differences in the population characteristics of the discovery cohort and of the replication cohorts, including in the proportion of asthmatic patients and levels of total serum IgE. These differences might have affected our results, especially because the genetic background for increased levels of total IgE may differ in nonasthmatic healthy individuals and in asthmatic patients [44]. Nevertheless, analyses excluding asthmatic patients produced similar results with genome-wide significance for the *HLA-C* region, indicating the robustness of our findings.

As the mechanisms mediating the risk conferred by the *HLA-C* region remains to be found, future studies will identify the causal genes/variants within the susceptibility loci associated with levels of total serum IgE by fine-mapping and by investigating the biological link between rs3130941/HLA-C and regulation of IgE production.

In summary, we performed a GWAS showing positive results for total serum IgE levels for the first time in an Asian population. Association of a SNP in the *HLA-C* region with total serum IgE levels reached genome-wide significance in our meta-analysis involving a total of 3654 Japanese adults. We also demonstrated that some of the previously reported genetic associations with total serum IgE levels were replicated across ethnicities.

Materials and Methods

Ethical 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, the Hokkaido University School of Medicine, and the University of Fukui. Written informed consent was obtained from each participant in accordance with institutional requirements and the principles of the Declaration of Helsinki.

Study Participants

The discovery cohort (Tsukuba cohort) consisted of 1180 individuals of Japanese ethnicity (967 healthy volunteers and 213 patients with asthma). The healthy volunteers without pulmonary diseases such as asthma and COPD were originally recruited for a genetic study of pulmonary function from the general population who visited the Tsukuba Medical Center for an annual health checkup [45]. All the participants were asked about their respiratory health, medical history, lifestyle, and exposure to environmental irritants (eg, cigarette smoke, allergens, and air pollution) and underwent heart and lung auscultation. The patients with asthma were recruited for genetic analysis of asthma from the Tsukuba University Hospital and its affiliated hospitals [46]. Asthma was diagnosed by pulmonary physicians according to the American Thoracic Society criteria as previously described [47]. Specific serum IgE antibody was measured for both the healthy and the asthmatic groups with the multiple allergen simultaneous test (MAST)-26 chemiluminescent assay systems (Hitachi Chemical Company, Tokyo, Japan) [48]. 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 (>4.40 lumicount) to at least 1 of the 14 allergens.

To replicate our findings in the discovery cohort, we analyzed 2 independent Japanese cohorts. The first replication cohort (Hokkaido cohort) comprised 619 healthy volunteers and 491 asthmatic patients from the Hokkaido University Hospital and its affiliated hospitals. This population was originally recruited for a case-control genetic association study searching for susceptibility genes to asthma and atopy [49]. Serum-specific IgE to *Dermatophagoides species*, molds, pollen, and animal dander was measured by a radioallergosorbent test (RAST). Atopy was defined as a positive response (>0.70 UA/mL) to at least 1 of these allergens.

The second replication cohort (Fukui cohort) comprised 1275 healthy volunteers and 89 asthmatic patients. This population was originally recruited from workers and students of the University of Fukui for a study of the genetic epidemiology of allergic rhinitis [50]. Serum IgE antibody specific to Japanese cedar, house dust, orchard grass, ragweed mix, *Candida species*, or *Aspergillus species* was measured by RAST. Atopy was defined as a positive response (>0.70 UA/mL) to at least 1 of these allergens.

Genotyping

Genomic DNA was extracted from peripheral blood samples of all participants by an automated DNA extraction system (QuickGene-610L; Fujifilm, Tokyo, Japan). Genotyping of the Tsukuba cohort was carried out using the Illumina HumanHap550v3 BeadChip (Illumina, San Diego, CA, USA) for the healthy volunteers and the HumanHap610-Quad BeadChip for the asthmatic patients. The concordance rate between the genotypes determined by the Illumina HumanHap550v3 BeadChip and the Illumina HumanHap610-Quad BeadChip among 182 duplicated samples was 0.99998 [46]. Quality control checks for the SNPs were performed separately using PLINK version 1.07 software [51]. None of the healthy volunteers or the asthmatic patients were removed owing to a call rate for autosomal SNPs of <0.02. SNPs with a missing genotype rate >0.01, minor allele frequency <0.01, or Hardy-Weinberg equilibrium P value $<1.0 \times 10^{-6}$ were excluded, leaving 479,940 SNPs that were common to the 2 arrays for analysis. Raw data is available upon request.

Genotyping accuracy on the X chromosome is often lower than that on other chromosomes because of difficulties involving clustering algorithms, higher frequencies of chromosome anomalies, and more missing data on X chromosome variants [52]. Genotyping of the pseudoautosomal region shared with the Y chromosome and hemizygous males can also be problematic. These analytic complexities could reduce the power of X chromosome analyses, making detection of reliable associations difficult. Therefore, in the current study, we decided to exclude the X chromosome from the analysis.

We imputed the genotypes of missing SNPs by using MACH version 1.0 software [53] to improve the resolution of candidate regions identified as associated with total IgE levels at *P* values $<1\times10^{-5}$. MACH employs a Markov chain algorithm and imputes missing genotypes by taking phased haplotypes as templates. We used 1000 Genomes Project data of Asian origin (JPT+CHB) (http://www.sph.umich.edu/csg/abecasis/MACH/download/1000G-2010-06.html) as the reference panel. To evaluate missing genotypes, we used 50 iterations of the Markov sampler to ensure reliable results.

To obtain the genomic inflation factor, we performed the multidimensional scaling (MDS) method using PLINK version 1.07 software. The MDS method is widely used in stratification methods, matching cases to controls based on genotype information (identity-by-state), resulting in discrete strata of individuals that can be analyzed using the Cochran–Mantel–Haenszel test [51].

For replication analyses of the original GWAS data, to obtain high-confidence results, we selected SNPs that are available in the ready-to-use predesigned TaqMan® SNP Genotyping assays (Applied Biosystems, Foster City, CA, USA) in each candidate region that satisfied the following conditions in the discovery Tsukuba cohort: (1) in strongest LD with the SNP most significantly associated with total IgE levels and (2) minor allele frequency >0.15. All assays are quality control tested using a mass spectrophotometer to verify sequence and yield. All assays have 1 VIC® and 1 FAM™ dye-labeled probe and 2 target-specific primers and undergo bioinformatics evaluation of target SNP sequences.

Validation of Association of Previously Reported Genes

A literature search was conducted in PubMed of publications up to June 1, 2013 on genetic association studies of total serum IgE levels. Keywords in the search strategy were ("IgE level" or "IgE concentration" or "serum IgE") and ("polymorphism" or "SNP" or "genetics") and ("association"). The search was restricted to human studies written in English. We reviewed the titles, abstracts, and texts of the publications to identify positive genetic association studies. Review articles and studies analyzing antigen-specific IgE production were excluded, as were studies using linkage analysis and transmission disequilibrium tests. We selected only genetic association studies. The references of the collected articles were also screened to find additional matching studies. From the retrieved publications, we selected eligible genes that were reported in 3 or more independent association studies or demonstrated by at least 1 GWAS so that we could as far as possible exclude potentially false-positive findings.

Because LD structures may be quite different between Japanese and Caucasian populations, we attempted gene-level replication instead of SNP-level replication. From the primary GWAS data, we chose the SNP with the strongest statistical evidence in a region extending +/−10 kilobases (kb) from each literature-selected candidate gene. The significance level was corrected for multiple testing using the SNPSpD program [16], which corrects for multiple testing of SNPs in LD with each other on the basis of the spectral decomposition of matrices of pairwise LD between SNPs. This method provides a useful alternative to more computationally intensive permutation tests.

Statistical Analysis

In the primary GWAS cohort, associations of genotypes of all the SNPs with log-transformed (base 10) levels of total serum IgE were analyzed by multiple linear regression models in PLINK version 1.07. Because total serum IgE levels are influenced by age, sex, smoking status, and asthma affection status [8,54], the original GWAS of the total serum IgE levels in the current study was adjusted according to these variables. Quantile-quantile plots and genomic inflation factors were calculated in PLINK version 1.07. In the replication studies, the associations were examined by the same methods in the Hokkaido cohort. As smoking behavior was not available in the Fukui cohort, the associations were adjusted only for age, sex, and asthma affection status in this cohort. Replication was declared only if $P<0.05$ and the direction of the effect was the same as in the primary GWAS. Combined analysis of the primary GWAS with the replication studies was performed by the basic meta-analysis function in PLINK version 1.07. Random-effect meta-analysis *P* values were estimated. We used the Haploview 4.2 program [55] to analyze the LD values between SNPs.

Supporting Information

Figure S1 Study flow chart. GWAS for total IgE levels was performed, followed by replication studies and meta-analysis. Validation of previously reported genes for IgE was also conducted using the GWAS data.
(TIFF)

Figure S2 Fine-mapping association plots on chromosome 6p21. Three peaks are identified: the MHC class I, MHC class II, and *LEMD2* regions.
(TIFF)

Figure S3 Fine-mapping association plots in the MHC class I region. The color of each circle reflects the LD (r^2) between a particular SNP and rs3130941 indicated as a purple diamond.
(TIFF)

Table S1 Results of meta-analysis for nonasthmatic healthy individuals only.
(DOCX)

Table S2 Results of meta-analysis after inclusion of atopic status as a covariate.
(DOCX)

Table S3 Previously reported polymorphisms significantly associated with total serum IgE.
(DOCX)

Table S4 Genetic influences of SNPs in the MHC class I/II regions on the association between rs3130941 and total IgE levels.
(DOCX)

Acknowledgments

We would like to thank Ms Flaminia Miyamasu, an associate professor at the Faculty of Medicine, University of Tsukuba, who proofread and commented on this paper. We also thank Ms Takako Nakamura for technical assistance with genotyping.

Author Contributions

Conceived and designed the experiments: YY TS NH. Performed the experiments: YY HM TS YK TH. Analyzed the data: YY HM TS EN TH MT NH. Contributed reagents/materials/analysis tools: YK HY HI TN YI TT SF SK MN. Wrote the paper: YY TS NH.

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Allergology International. 2014;63:27-35
DOI: 10.2332/allergolint.13-OA-0579

ORIGINAL ARTICLE

Lung Functions of Japanese Patients with Chronic Rhinosinusitis Who Underwent Endoscopic Sinus Surgery

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ABSTRACT

Background: Chronic rhinosinusitis (CRS), which is clinically classified into CRS without nasal polyps (CRSsNP) and CRS with nasal polyps (CRSwNP), shows considerable geographic differences and heterogeneity. Eosinophilic (E) CRS with nasal polyps (ECRSwNP) has a higher degree of disease severity and higher frequency of comorbid asthma. Epidemiologic studies in different ethnic populations have improved understanding of the pathophysiology of the disease. Here we report the clinical characteristics of Japanese patients with medically refractory CRS undergoing endoscopic sinus surgery (ESS).

Methods: We recruited a total of 210 CRS patients and assessed them by nasal endoscopy, the Lund-Mackay score using computed tomography (CT), peripheral eosinophilia and smoking status. We also examined the comorbidity of asthma, effects of age and lung functions in the patients.

Results: In this study, 13% of CRSwNP patients and 20% of CRSwNP patients with peripheral blood eosinophilia exhibited obstructive lung dysfunction (FEV_1/FVC <70%) despite the absence of an asthma diagnosis. Among elderly nonsmoker patients (≥ 60 years) who had never been diagnosed with asthma, 50% of CRSwNP patients with peripheral blood eosinophilia showed decreased FEV_1/FVC <70%.

Conclusions: Our findings suggest that asthma is under-diagnosed in CRS patients who undergo ESS, especially the elderly. Although the association between CRS and asthma has been recognized, increased attention to the comorbidity of obstructive airway diseases such as asthma is still needed for management of medically refractory CRS.

KEY WORDS

asthma, chronic rhinosinusitis, eosinophils, lung functions, nasal polyps

INTRODUCTION

Chronic rhinosinusitis (CRS), a common disease associated with persistent inflammation of the nasal and paranasal sinuses, is a public health problem resulting in a socioeconomic burden throughout the world.[1,2] CRS is commonly classified into two groups, CRSsNP and CRSwNP. Considerable heterogeneity within CRSwNP has been recognized and there are geographic differences in the condition.[3,4] Tissue from Caucasian patients with CRSwNP is characterized by eosinophilic inflammation, whereas samples from Asian patients are biased toward neutrophilic inflammation.[3,4] Eosinophilic (E) CRSwNP has been found in 65-90% of subjects with CRSwNP in Caucasians and in 50% of them in East Asian populations.[1-3] ECRSwNP represents a higher degree of disease severity, with an impaired sense of smell, higher recurrence rate after surgery and higher frequency of comorbid asthma.[2] The association of ECRSwNP and asthma is well recognized in western countries; [5,6] however, asthma comorbidity in CRSwNP patients in

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Conflict of interest: No potential conflict of interest was disclosed.
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Received 17 May 2013. Accepted for publication 25 July 2013.

Table 1 Clinical characteristics of patients with chronic rhinosinusitis

	CRSsNP $n = 40$	CRSwNP $n = 170$	*P*-value
Age (median, range)	57 (35-64)	54.5 (44-63)	NS
Sex (male/female) (female %)	31/9 (23)	109/61 (36)	NS
Smoker, no. (%)	21 (53)	56 (33)	<0.05
Current smoker, no. (%)	21 (53)	46 (27)	<0.01
Brinkman index of smokers	430 (290-728)	560 (300-854)	NS
Asthma, no. (%)	2 (5)	44 (26)	<0.01
Aspirin-induced asthma, no. (%)	0 (0)	10 (6)	NS
Allergic rhinitis, no. (%)	8 (20)	48 (28)	NS
Rhinorrhea, no. (%)	34 (85)	124 (73)	NS
Nasal congestion, no. (%)	23 (58)	138 (81)	<0.01
Headache, no. (%)	17 (43)	38 (22)	<0.05
Olfactory dysfunction, no. (%)	11 (28)	112 (66)	<0.0001

P values were determined using the Mann-Whitney U test, or Fisher's exact test as appropriate. CRSwNP, chronic rhinosinusitis with nasal polyps; CRSsNP, chronic rhinosinusitis without nasal polyps; NS, not significant.

Asian countries has not been fully studied. Nor are the clinical features of CRS such as sinus scores evaluated by CT scanning and lung functions in Asian populations well investigated.

Epidemiologic studies have shown that asthma and rhinitis often coexist in the same patients and suggest the 'united airways' concept.[4,7-11] Underdiagnosis and undertreatment of asthma is a significant public problem all over the world, especially in the elderly.[12] The awareness of asthma is frequent in those with comorbid rhinitis,[13] and it has also been suggested that symptoms may predominate in one organ and be unrecognized in other organs even though they exist.[8] A higher CT score and more nasal polyp formation are observed in elderly patients with CRS.[14] However, the influence of aging on the clinical features of CRS in Japanese patients has not been examined.

ESS is the treatment choice for medically refractory CRS with or without nasal polyposis.[15] To clarify the clinical features of refractory CRS in Japanese patients, we conducted a cross-sectional study with a total of 210 CRS patients who underwent ESS. We assessed their clinical phenotypes through the use of nasal endoscopy, considering peripheral eosinophilia, CT scores based on the Lund-Mackay system, smoking status, comorbidity of asthma, effects of age and lung functions.

METHODS

SUBJECTS

CRS was defined as a condition with at least two of the following symptoms: anterior and/or posterior rhinorrhea, nasal obstruction, decreased sense of smell, and nasal pressure existing for 12 weeks despite medical management.[1,2] We recruited all patients with CRS who underwent ESS at the University of Yamanashi Hospital from January 2002 to October 2011. All individuals were Japanese, and we excluded patients with autoimmune disease, cancer, papilloma, fungal infection, postoperative maxillary cysts, choanal polyps, and nasal foreign bodies before enrollment. We excluded patients with allergic fungal rhinosinusitis based on CT and/or histopathological findings from the start. We included patients with unilateral CRS and those with nasal polyps. The presence or absence of nasal polyps was confirmed by endoscopy. Finally, a total of 210 subjects were analyzed (Table 1). CRS patients were classified into CRSwNP and CRSsNP groups based on the criteria of the American Academy of Otolaryngology-Head and Neck Surgery Chronic Rhinosinusitis Task Force.[1] Comorbidity of asthma was determined from the patients' medical histories based on doctors' interviews. We defined individuals who had asthma diagnosed by a doctor at any point in their lifetime as having bronchial asthma. This study was approved by the ethics committee of the University of Yamanashi Hospital. We informed patients that any clinical data would be used for research analyses by placing a notice on walls in the medical examination room and hospital lobby in the University of Yamanashi Hospital. We individually responded to patients who dissented from such use. In this study, we recruited subjects who did not dissent from use of the clinical data. This process of obtaining informed consent was approved by the ethics committee of the University of Yamanashi Hospital.

PERIPHERAL EOSINOPHIL COUNT

A total of 19 patients (9%) were treated with systemic corticosteroids in the two months before the operation, and 91 patients (43%) were treated with intranasal steroids in the month before the operation. Since the eosinophil count is sensitive to steroid treatment, we recorded the highest peripheral blood eosinophil percentage among all the blood tests done before operation.

LUNG FUNCTION AND COMPUTED TOMOGRAPHY

Spirometry was performed for all patients using a DISCOM 21 FXII spirometer (CHEST MI., Inc., Tokyo, Japan). Forced expired volume in one second (FEV_1)/forced vital capacity (FVC) (FEV_1%) was calculated. Maximum expiratory flow rates at 50% and 25% of the FVC (V50 and V25) were obtained from flow/volume curves. FEF25-75 and lung functions after application of a bronchodilator were not available for this study. CT scans of the sinuses were available for 54 patients with CRSwNP and were graded based on the Lund-Mackay staging system.[16] We used the average of the right and left side scores in the following analyses.

HISTOLOGICAL ANALYSIS

It is well known that infiltration of eosinophils in nasal polyps is a characteristic of ECRSwNP. However, it remains unclear whether eosinophilic infiltration exists in nasal mucosal tissue of CRS without nasal polyp involvement. To assess eosinophil infiltration in sinonasal mucosal tissues without polyps, we examined the maxillary mucosa histologically. Among the 210 CRS patients enrolled in this study, non-polyp mucosal tissues from the maxillary sinuses were obtained from 46 CRSwNP patients. Paraffin-embedded sections were stained with hematoxylin and eosin, and the number of infiltrating eosinophils were counted in four randomly selected high power (×400) magnification fields.

STATISTICAL ANALYSIS

Demographic data, lung functions, and CT scores were compared using the Mann-Whitney U test or Fisher's exact test. The correlational validity of the peripheral blood eosinophil percentage with the mucosal eosinophil count was assessed using Spearman rank correlations. Statistical significance was set at $P < 0.05$. All statistical analyses were performed using R (R Development Core Team, http://www.r-project.org/).

RESULTS

SUBJECTS' CHARACTERISTICS

Of the 210 CRS patients who underwent ESS, 40 (19%) had CRSsNP and 170 (81%) had CRSwNP. Clinical features of each group are shown in Table 1. Current smokers were more frequent in the CRSsNP group (53%) than in the CRSwNP group (27%). The comorbidity of asthma was significantly higher for CRSwNP (26%) than for CRSsNP (5%). Among subjective symptoms, olfactory dysfunction was more frequent in CRSwNP (66%) than in CRSsNP (28%).

Recent studies have shown that the blood eosinophil percentage is the most accurate clinical factor to distinguish the eosinophilic type of CRSwNP from the non-eosinophilic type;[17,18] however, precise clinical diagnostic criteria including the peripheral eosinophil percentage for ECRSwNP have not been determined. In this study, we divided the patients with CRSwNP into two groups to clarify the clinical characteristics of each group. Since the median value of the eosinophil percentage of the total leukocyte count in subjects with CRSwNP was 5%, we defined subjects with an eosinophil percentage <5% as having CRSwNP without peripheral blood eosinophilia and subjects with ≥5% as having CRSwNP with peripheral blood eosinophilia. A total of 87 subjects (51%) were classified into the CRSwNP group without peripheral blood eosinophilia, and 83 subjects (49%) were classified into the group having CRSwNP with peripheral blood eosinophilia (Table 2). The comorbidity of asthma was significantly higher for CRSwNP with peripheral blood eosinophilia (43%) than for CRSwNP without peripheral blood eosinophilia (9%), and the comorbidity of aspirin-induced asthma (AIA) or allergic rhinitis was significantly higher for CRSwNP with peripheral blood eosinophilia than for the CRSwNP without peripheral blood eosinophilia (Table 2). Olfactory dysfunction was also frequently observed in the group having CRSwNP with peripheral blood eosinophilia (Table 2).

CT SCORES IN PATIENTS WITH CRSwNP

The Lund-Mackay score is commonly used for assessment of the stage and severity of CRS.[16] We examined the severity of the disease in subjects with CRSwNP using CT scores based on the Lund-Mackay system. The total score for CRSwNP with peripheral blood eosinophilia had a tendency to be higher than that for CRSwNP without peripheral blood eosinophilia (Fig. 1) but the difference was not statistically significant ($P = 0.051$). There was no significant difference between CRSwNP without peripheral blood eosinophilia and CRSwNP with peripheral blood eosinophilia for the maxillary sinus score, sphenoid sinus score, frontal sinus score, and ostiomeatal complex score (data not shown). However, the anterior ethmoid (AE) sinus score and posterior ethmoid (PE) sinus score of CRSwNP with peripheral blood eosinophilia were significantly higher those for CRSwNP without peripheral blood eosinophilia ($P < 0.05$ and < 0.001, respectively) (Fig. 1).

CORRELATION BETWEEN EOSINOPHIL INFILTRATION IN SINONASAL MUCOSA AND PERIPHERAL BLOOD EOSINOPHIL COUNT IN PATIENTS WITH CRSwNP

Infiltration of eosinophils in nasal polyps is a characteristic of ECRSwNP. Since whether eosinophilic infiltration exists in nasal mucosal tissue of CRS without nasal polyp involvement remains unknown, we investigated the correlation between blood and mucosal eosinophilia in a total of 46 patients with CRSwNP. The count of mucosal eosinophils strongly correlated

Table 2 Clinical characteristics of patients with CRSwNP

	CRSwNP (n = 170)		P-value
	without peripheral blood eosinophilia n = 87	with peripheral blood eosinophilia n = 83	
Age (median, range)	58 (45-66)	52 (44-61)	NS
Sex (male/female) (female %)	54/33 (38)	55/28 (34)	NS
Smoker, no. (%)	31 (36)	25 (31)	NS
Current smoker, no. (%)	25 (29)	21 (26)	NS
Brinkman Index of smokers (median, range)	730 (330-900)	460 (293-620)	NS
Asthma, no. (%)	8 (9)	36 (43)	<0.000001
Aspirin-intolerant asthma, no. (%)	1 (1)	9 (11)	<0.01
Allergic rhinitis, no. (%)	16 (18)	32 (39)	<0.01
Rhinorrhea, no. (%)	64 (74)	60 (72)	NS
Nasal congestion, no. (%)	70 (81)	68 (82)	NS
Headache, no. (%)	23 (26)	15 (18)	NS
Olfactory dysfunction, no. (%)	49 (56)	63 (76)	<0.01

P values were determined using the Mann-Whitney U test or Fisher's exact test as appropriate. NS, not significant.

with the peripheral blood eosinophil percentage (R = 0.67, $P < 0.000001$) (Fig. 2).

LUNG FUNCTIONS OF THE CRS PATIENTS

The close relationship between asthma and CRS is widely recognized. We therefore assessed lung functions of the CRS patients. We first excluded nine patients whose smoking histories were unknown from the analysis. FEV_1/FVC was significantly lower in subjects with CRSwNP than in subjects with CRSsNP, and was also lower in the group having CRSwNP with peripheral blood eosinophilia than in the group having CRSwNP without peripheral blood eosinophilia (Fig. 3a, Table 3). There were 18 patients being treated with corticosteroids among the subjects whose lung functions were assessed. One patient with CRSsNP (2.6%), two patients with CRSwNP without peripheral blood eosinophilia (2.4%) and 15 patients who had CRSwNP with peripheral blood eosinophilia (19%) had received systemic corticosteroid treatment prior to surgery. Although there were more patients receiving corticosteroids in the group having CRSwNP with peripheral blood eosinophilia than in the groups having CRSsNP and CRSwNP without peripheral blood eosinophilia, FEV_1/FVC was significantly lower in the subjects who had CRSwNP with peripheral blood eosinophilia than in the subjects of the other two CRS groups. Furthermore, %V25 was lower in the CRSwNP group than in the CRSsNP group, and both %V25 and %V50 were lower for CRSwNP with peripheral blood eosinophilia than for CRSwNP without peripheral blood eosinophilia (Table 3).

Decreased FEV_1/FVC is a clinical feature of obstructive lung diseases such as bronchial asthma and chronic obstructive pulmonary disease (COPD). Since smoking is the leading cause of COPD, we next examined the influence of smoking status on lung functions of subjects with CRSsNP and CRSwNP. There was no significant difference in lung functions (FEV_1/FVC, %V25, and %V50) between current smokers, former smokers (smokers) and never smokers (nonsmokers) among the entire group with CRS (Fig. 3b, Table 3). After excluding current and former smokers, FEV_1/FVC was lower in the CRSwNP group than in the CRSsNP group, and was lower in the CRSwNP group with peripheral blood eosinophilia than in the CRSwNP group without peripheral blood eosinophilia (Fig. 3c). Both %V25 and %V50 were lower in patients who had CRSwNP with peripheral blood eosinophilia than in those who had CRSwNP without peripheral blood eosinophilia (Table 3).

We next stratified the CRS patients who had never been diagnosed with asthma prior to ESS by the comorbidity of asthma, and focused on their lung functions. Since obstruction has been traditionally defined by an FEV_1/FVC ratio of less than a certain percentage, usually 70 to 75%,[19] we divided subjects into three classes according to their lung function: FEV_1/FVC <70%, 70%≤ FEV_1/FVC <75% and FEV_1/FVC ≥75% (Fig. 4). In 13% of patients with CRSwNP who had never been diagnosed as having asthma, FEV_1/FVC was less than 70% (Fig. 4a). Furthermore, 20% of patients having CRSwNP with peripheral blood eosinophilia had FEV_1/FVC of less than 70% despite the absence of a previous asthma diagnosis (Fig. 4a). The same tendency was observed even after excluding current and former smokers, and smoking status did not influence the obstructive lung dysfunction in subjects who had CRSwNP and CRSwNP with peripheral blood eosinophilia (Fig. 4b).

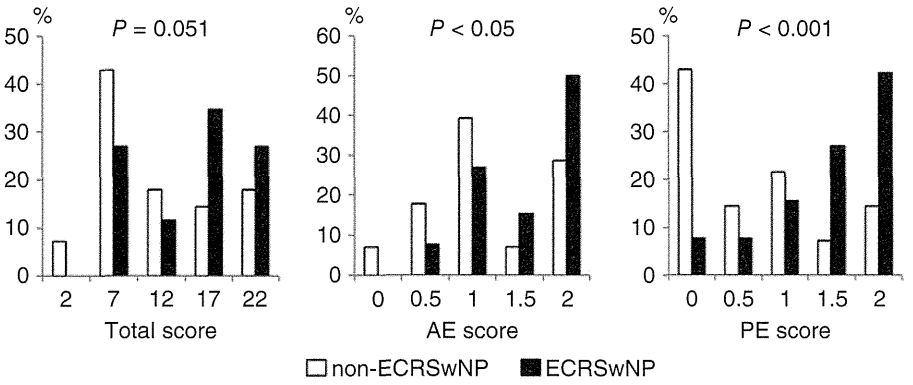

Fig. 1 Comparison of CT scores for CRSwNP with and without peripheral blood eosinophilia. CT scores based on the Lund-Mackay staging system were compared between subjects who had CRSwNP without peripheral blood eosinophilia (n = 28) and CRSwNP with peripheral blood eosinophilia P (n = 26). P values were determined using the Mann-Whitney U test. AE score, anterior ethmoid sinus score; PE score, posterior ethmoid sinus score.

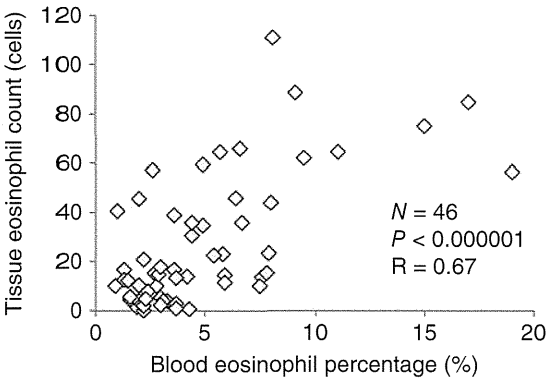

Fig. 2 Correlation between percentage of eosinophils in peripheral blood and the tissue eosinophil count in the mucosa of the maxillary sinus. The tissue eosinophils of patients with CRSwNP (n = 46) were counted at high power maginification (×400). Correlation validity was assessed by using Spearman rank correlations.

Underdiagnosis of asthma is an important problem, especially in the elderly.[12] Therefore we further examined whether there were age-related differences in the prevalence of each subgroup or in FEV_1/FVC. We divided nonsmoker patients who had never been diagnosed with asthma into nonelderly (<60 years) and elderly groups (≥60 years). In the elderly group, obstructive lung dysfunction (FEV_1/FVC <70%) was more frequently seen in those with CRSwNP (28%) than in those with CRSsNP (0%), and for CRSwNP with peripheral blood eosinophilia (50%) than for CRSwNP without peripheral blood eosinophilia (18%) (Fig. 4c).

DISCUSSION

There is considerable heterogeneity within the CRSwNP subgroup,[3,4] and importance of understanding CRS within the context of racial and ethnic populations has been suggested.[20] This study revealed the clinical characteristics of Japanese patients with medically refractory CRS who underwent ESS. Epidemiologic studies have reported that rhinitis and asthma often coexist in the same patients.[1,5,6,8] The 'united airways' concept implies that there is a link between upper and lower airway inflammation.[8,9,21] A recent study reported the asthma comorbidity in patients with CRSwNP who underwent ESS (n = 19) to be 32% in Japanese patients.[22] In the United States, the frequencies of comorbidity of asthma were reported to be 11% in those with CRSsNP and 44% in those with CRSwNP.[23] In this study, the frequency was 26% for CRSwNP, and it was significantly higher for CRSwNP with peripheral blood eosinophilia (43%) than for CRSwNP without peripheral blood eosinophilia (9%). The frequency of comorbidity of asthma observed in the group having CRSwNP with peripheral blood eosinophilia (43%) in our study was similar to that in the CRSwNP group (44%) in the United States. Furthermore, it has been reported that ECRSwNP has a higher degree of disease severity in western countries.[1] AIA represents a severe phenotype of asthma and we found significantly higher comorbidity of AIA for CRSwNP with peripheral blood eosinophilia than for the CRSwNP without peripheral blood eosinophilia in this study.

A recent report has shown that CRS patients with anosmia have a higher density of eosinophils infiltrating the olfactory epithelium, and exhibit more abnormalities on CT and endoscopic examination, including being more likely to exhibit nasal polyposis than other CRS patients.[24] We observed olfactory dysfunction with high frequency in subjects with CRSwNP (66%), especially in those who had CRSwNP with peripheral blood eosinophilia (76%). Our findings support previous research results. CT scanning is useful

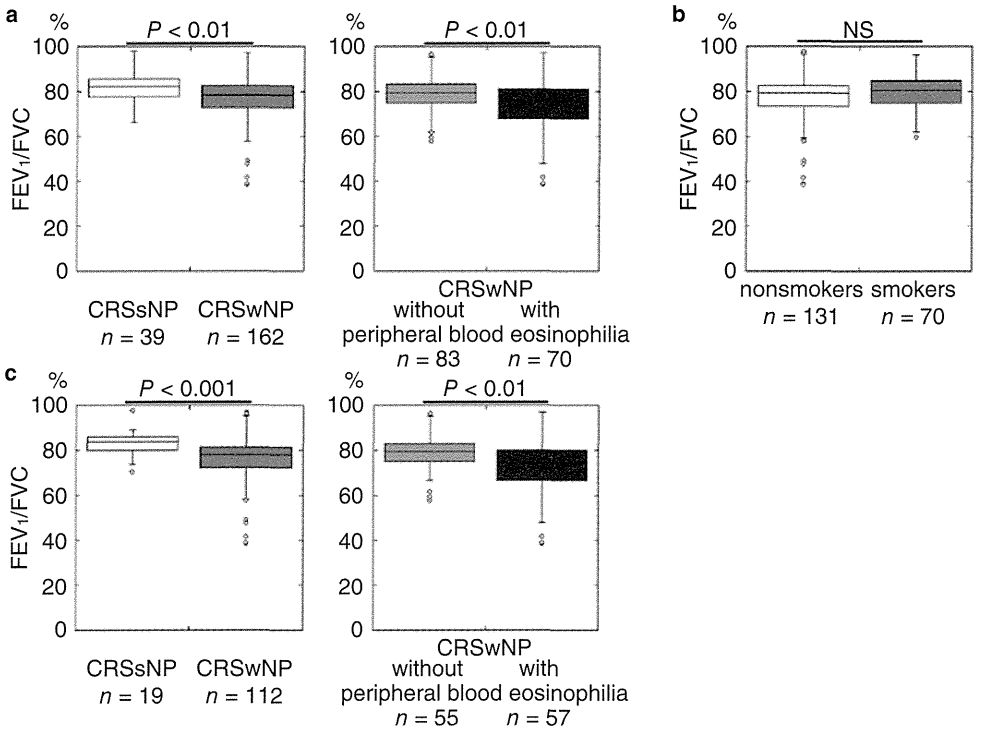

Fig. 3 Comparison of FEV_1/FVC ratios among subgroups of CRS. **a**) Comparison between CRSsNP and CRSwNP, and between CRSwNP with and without peripheral blood eosinophilia. **b**) Comparison between nonsmokers and (current and former) smokers among the entire group with CRS. **c**) Comparison between CRSsNP and CRSwNP, and between CRSwNP with and without peripheral blood eosinophilia after excluding current and former smokers. Rectangles include the range from the 25th to 75th percentiles, horizontal lines indicate the median and vertical lines indicate the 10th to 90th percentiles. *P* values were determined using the Mann-Whitney U test. FVC, forced vital capacity; FEV_1, forced expired volume in one second.

Table 3 Lung functions of subgroups of CRS

	CRS		*P*-value	Nonsmokers with CRS		*P*-value
	CRSsNP (*n* = 39)	CRSwNP (*n* = 162)		CRSsNP (*n* = 19)	CRSwNP (*n* = 112)	
%FVC	105.0 (93.5-111.8)	106.7 (95.2-118.3)	NS	102.0 (88.2-110.9)	107.8 (95.3-118.5)	<0.05
$\%FEV_1$	102.5 (95.7-109.7)	101.5 (89.6-110.8)	NS	106.5 (94.0-111.2)	103.3 (88.5-112.1)	NS
FEV_1/FVC	82.3 (77.4-85.6)	78.4 (72.5-82.6)	<0.01	83.9 (80.2-86.3)	78.1 (72.4-81.4)	<0.001
%V50	68.5 (61.4-85.7)	67.3 (45.7-83.9)	NS	68.4 (60.7-76.1)	64 (43.8-81.9)	NS
%V25	58.8 (44.2-66.2)	42.7 (29.3-55.3)	<0.001	60.8 (52.7-66.2)	42.7 (27.7-54.5)	<0.001
	CRSwNP		*P*-value	Nonsmokers with CRSwNP		*P*-value
	without peripheral blood eosinophilia P (*n* = 83)	with peripheral blood eosinophilia (*n* = 79)		without peripheral blood eosinophilia (*n* = 55)	with peripheral blood eosinophilia (*n* = 57)	
%FVC	106.4 (94.7-113.2)	108.2 (97.5-121.0)	NS	106.7 (95.1-113.2)	110.3 (97.6-121.6)	NS
$\%FEV_1$	101.8 (91.9-112.9)	101.5 (80.1-108.5)	NS	105.1 (92.6-114.4)	98.2 (78.1-108.8)	NS
FEV_1/FVC	79.5 (74.7-83.3)	75.3 (67.8-81.2)	<0.01	79.4 (75.1-83.2)	74.4 (66.7-80.1)	<0.01
%V50	69.6 (55.2-85.7)	58.5 (37.7-81.7)	<0.01	68.3 (57.3-84.7)	53.5 (36.8-74.0)	<0.01
%V25	45.6 (36.8-59.5)	40.9 (25.8-51.4)	<0.01	45.1 (37.5-56.3)	38.7 (22.4-48.4)	<0.01

Lung functions are expressed as percentages of predicted values and are presented as medians (interquartile ranges). *P* values were determined using the Mann-Whitney U test. FVC, forced vital capacity; FEV_1, forced expired volume in one second; V50 and V25 of FVC, maximum expiratory flow rates at 50% and 25%; $FEV_1\%$, FEV_1/FVC %.

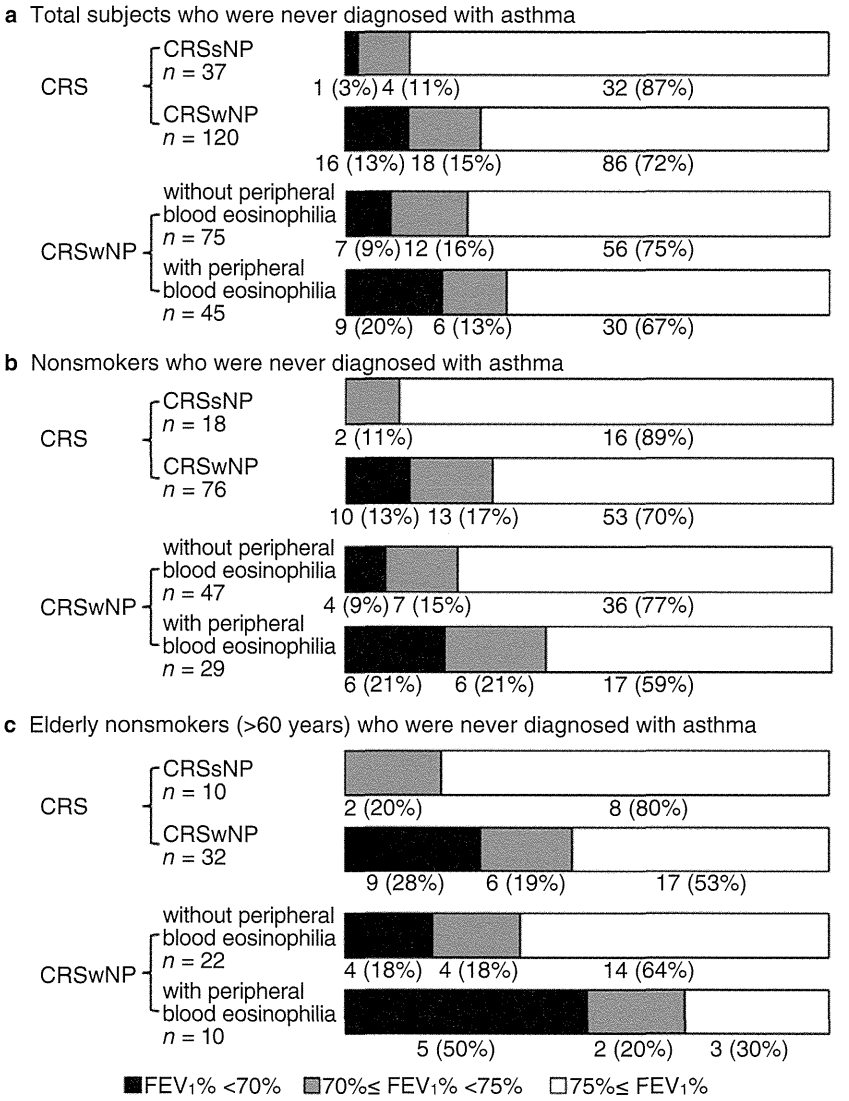

Fig. 4 Obstructive lung dysfunctions of CRS patients who had never been diagnosed with asthma prior to ESS. **a-c**) Comparison between CRSsNP and CRSwNP, and between CRSwNP with and without peripheral blood eosinophilia. **a**) In total subjects. **b**) In subjects excluding current and former smokers. **c**) In elderly nonsmoker subjects (>60 years) who had never been diagnosed with asthma.

for the visualization of disease and enables us to determine the extent of rhinosinusitis accurately. The Lund-Mackay score evaluated on CT scans is widely used in assessment of CRS.[16] A recent study has shown that an increased blood eosinophil percentage and the CT image scores for the PE sinus and the olfactory cleft are good predictors of ECRSwNP.[18] In this study, although the total score for CRSwNP with peripheral blood eosinophilia did not significantly differ from that for CRSwNP without peripheral blood eosinophilia, the AE sinus and PE sinus scores of CRSwNP with peripheral blood eosinophilia were significantly higher than those for CRSwNP without peripheral blood eosinophilia. Thus, our results support previous findings on involvement of the posterior ethmoid sinus in ECRSwNP.

Infiltration and activation of eosinophils in nasal polyps is a characteristic of eosinophilic CRSwNP, and it has been reported that the peripheral blood eosinophil percentage is positively correlated with the number of eosinophils in nasal polyps.[25] In this study, we found eosinophilic infiltration in nasal mucosal tissue not involving nasal polyps and a positive correlation between blood and mucosal eosinophilia in patients with CRSwNP. Thus, it is necessary to be aware of the possibility of eosinophilic inflammation not only in nasal polyps but also in nasal mucosa for the treatment of CRSwNP patients with high blood

eosinophil levels.

A recent report showed a high prevalence of asymptomatic lower airway dysfunctions in patients with CRSwNP in the United Kingdom.[26] In this study, 28% of CRSwNP patients exhibited FEV_1/FVC of less than 75%, and 20% of patients who had CRSwNP with peripheral blood eosinophilia had FEV_1/FVC of less than 70% despite the absence of an asthma diagnosis. Decreased FEV_1/FVC (<70%) was more frequently observed in patients having CRSwNP with peripheral blood eosinophilia (21%) than in those having CRSwNP without peripheral blood eosinophilia (9%), even after excluding current and former smokers. Never smokers comprise a substantial proportion of patients with COPD; however, asymptomatic decreased lung function suggestive of an asthmatic phenotype was frequently observed in CRSwNP, especially in CRSwNP with peripheral blood eosinophilia. Most patients with asthma have symptoms of rhinitis, but in many cases symptoms may predominate in one organ and be unrecognized in other organs even though they exist.[8] Our findings also suggest the necessity of paying increased attention to the possible comorbidity of obstructive airway diseases such as asthma for management of refractory CRS.

The underdiagnosis and undertreatment of asthma are serious problems throughout the world,[13,27-30] especially in the elderly.[29] About half of elderly people with asthma have not been diagnosed, and the underuse of objective testing such as spirometry has been considered to be one reason.[30] In this study, after excluding patients who had ever been diagnosed with asthma and current or former smokers, decreased FEV_1/FVC (<70%) tended to be more prevalent in elderly patients in the CRSwNP and CRSwNP groups with peripheral blood eosinophilia. Although FEV_1/FVC normally decreases with age and FEV_1/FVC lower than 70% might be a normal finding, an FEV_1/FVC ratio of less than 70% increases the probability of asthma in elderly patients with asthma symptoms.[30] Careful assessment of asthma by means of systemic inquiries about respiratory symptoms and objective testing by spirometry seems to be necessary in subjects with refractory CRS, especially elderly patients. Early diagnosis and good asthma control are important to reduce morbidity and healthcare costs as well as minimize the development of chronic illnesses,[13] and appropriate diagnosis and management of asthma would contribute to mitigating the severity of their CRS.

In conclusion, we found that 19% of subjects with CRS who underwent ESS had CRSsNP, and 81% CRSwNP. We confirmed that both the AE and PE sinus CT scores of the Lund-Mackay staging system were helpful for identifying CRSwNP with peripheral blood eosinophilia. Obstructive lung dysfunctions are frequently observed in CRSwNP with peripheral blood eosinophilia, especially in elderly persons, despite the absence of an asthma diagnosis. Although further studies are needed, our findings will contribute to better understanding of the pathophysiology of CRS.

ACKNOWLEDGEMENTS

We thank all the patients for participating in the study as well as the collaborating physicians for collecting samples. We would like to thank Chizuru Nagagata and Yuko Hiraoka for data collection. We thank K. Barrymore for proofreading this document.

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ORIGINAL ARTICLE

Risk Factors for Eosinophilic Otitis Media in Patients with Eosinophilic Chronic Rhinosinusitis

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Objective: This study aimed to determine risk factor(s) for developing eosinophilic otitis media (EOM) in patients with eosinophilic chronic rhinosinusitis (ECRS) associated with bronchial asthma.

Materials and Methods: A total of 47 patients with ECRS and bronchial asthma were divided into two groups: 31 patients with EOM (EOM(+) group) and 16 patients without EOM (EOM(-)group). We investigated the clinical characteristics, including eustachian tube (ET) function, based on results of sonotubometry and responses to a questionnaire in both groups. Sonotubometry was also performed in 12 healthy controls.

Results: There were no significant differences between the two groups regarding clinical characteristics, except for age at onset of the diseases. The average ages at onset of bronchial asthma and rhinosinusitis in the EOM(+) group were almost 10 years earlier than those in the EOM(-) group. The opening duration of the ET measured by sonotubometry in the EOM(+) group and EOM(-) group was significantly longer than that in the control group (p=0.009 and p=0.035, respectively). In addition, the incidence of symptoms suggesting insufficiency of tubal closing (Habitual sniffing*, Autophony*, Echo to an ambient sound**, Feeling of ear stuffiness**) was significantly more frequent in the EOM(+) group than in the EOM(-) group (*p<0.05, ** p<0.01).

Conclusion: Patients with ECRS associated with bronchial asthma are at risk of developing EOM with insufficient closing of the ET. Repeated actions inducing positive pressure on the nasopharyngeal ostium of the ET, such as blowing the nose and nasal irrigation, might lead to the development of EOM.

Submitted : 30 July 2013 *Revised : 20 September 2013* *Accepted : 25 September 2013*

Introduction

There are some reports of patients with intractable otitis media with effusion or chronic otitis media associated with bronchial asthma[1-7]. These patients exhibit clinical characteristics that are markedly different from those of patients with common otitis media. In 1997, intractable otitis media associated with bronchial asthma was named "eosinophilic otitis media" (EOM) because middle ear effusion contains numerous eosinophils, regardless of the presence of type I allergy [1]. Diagnostic criteria of EOM were established in 2011 and are shown in Table 1 [3]. Minor criteria include an association with bronchial asthma and nasal polyposis because there is a significantly higher prevalence of these diseases in EOM patients than in common otitis media patients. Chronic rhinosinusitis with nasal polyps is frequently observed in patients with bronchial asthma, with an incidence of greater than 50% [8]. In particular, most patients with aspirin-intolerant asthma show multiple nasal

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polyposis. These conditions are called eosinophilic chronic rhinosinusitis (ERCS) because massive accumulation of eosinophils occurs in the polyps and sinus mucosa [9]. However, the cause of EOM in these patients has not yet been clarified. Some patients with ECRS develop EOM and others do not. Iino et al. [4] suggested that one possible factor for developing EOM was the presence of a patulous eustachian tube (ET). The presence of ECRS may alter ET function.

In the present study, we evaluated clinical characteristics, including ET function, in patients with ECRS with bronchial asthma with and without EOM, and identified the predominant factors leading to the development of EOM.

Materials and Methods

Patients

Forty-seven patients with ECRS associated with bronchial asthma were included in this study. They were diagnosed as having ECRS because, histologically, they all showed nasal polyposis with eosinophilic-dominant infiltration in the nasal polyps. We divided them into two groups: patients with EOM and those without EOM. The diagnosis of EOM in each patient was made on the basis of the criteria proposed by Iino et al. [3] as shown in Table 1. Patients with Churg-Strauss syndrome were excluded from the study. A total of 31 patients (62 ears) with EOM (EOM(+) group) and 16 patients (32 ears) without EOM (EOM(-) group) were evaluated on the basis of the following parameters: sex, age, computed tomography (CT) findings, peripheral blood eosinophil count (%), and total serum IgE level. ET function was evaluated by sonotubometry. A questionnaire was distributed to all of the patients. The questionnaire consisted of questions regarding ear and nasal symptoms that are associated with a patulous ET[10], age at onset of bronchial asthma, rhinosinusitis, and otitis media, a history of sinus operation, and use of nasal irrigation. All of the patients were treated with inhaled corticosteroids with or without systemic administration of corticosteroids for bronchial asthma, nasal corticosteroids for ECRS, and antileukotriene agents. Twelve healthy patients (24 ears) were also studied as controls for sonotubometry. None of the patients had medical histories involving significant body weight loss, pregnancy, or other conditions that affect the loss of fluid or adipose tissue around the peritubal lesion. Written informed consent was obtained from all of the patients prior to the study. The research and ethics committee of Jichi Medical University Saitama Medical Center approved this study.

Table 1. Diagnostic criteria of eosinophilic otitis media [3]

Major: Otitis media with effusion or chronic otitis media with eosinophil dominant effusion
Minor: 1. Highly viscous middle ear effusion
2. Resistance to conventional treatment for otitis media
3. Association with bronchial asthma
4. Association with nasal polyposis
Definitive case: Positive for major + two or more minor criteria
Exclusion criteria: Churg-Strauss syndrome, hypereosinophilic syndrome

CT image scoring

The extent of sinus disease identified by CT was evaluated using the Lund-Mackay scoring system [11]. To evaluate the sinus content, the five major right and left sinuses (frontal, maxillary, anterior and posterior ethmoids, and sphenoid) and the ostiomeatal complex were scored on a two-point scale as follows: 0, clear; 1, partial opacification; and 2, total opacification.

Sonotubometry

To evaluate ET function, sonotubometry (JK-05; RION Co, Ltd., Tokyo, Japan) was performed in all patients. In the EOM (+) group, we confirmed a lack of middle ear effusion and otorrhea in the mesotympanum by myringotomy and suctioning. After removal of rhinorrhea by suctioning, we measured the duration of eustachian tubal opening [4] induced by a sound pressure level change of >43 dB produced by voluntary dry swallowing when 60 dB of a 7-kHz bandpass sound was introduced through a nasal olive tip into one of the nostrils.

Statistical analyses

Statistical analyses were performed using the unpaired *t*-test or chi-square test. *p* values of less than 0.05 were considered statistically significant.

Results

Clinical characteristics

Clinical features of the patients in the three groups are shown in Table 2. There were no significant differences in sex and age among the three groups. Other parameters, such as CT score, peripheral eosinophil percentage, and serum IgE level showed no significant differences between the EOM(+)and the EOM(-) groups.

Responses to the questionnaire

The average ages at onset of bronchial asthma, rhinosinusitis, and otitis media in the EOM (+) group are shown in Table 3. The average ages at onset of bronchial asthma and rhinosinusitis in the EOM(+) group were almost 10 years earlier than those in the EOM(-) group. Most of the patients were affected by rhinosinusitis or bronchial asthma first, and otitis media developed more than 10 years later.

The incidence of symptoms suggesting the presence of a patulous ET (Habitual sniffing*, Autophony*, Echo to an ambient sound**, Feeling of ear stuffiness**) was significantly more frequent in the EOM(+) group than in the EOM(-) group (*p<0.05, ** p<0.01) (Table 4). In addition, most of the patients had undergone nasal irrigation, but 10 patients out of 13 in the EOM(+) group stopped nasal irrigation because of deterioration of their EOM after irrigation. In contrast, all 13 patients in the EOM(-) group, except for one, continued nasal irrigation.

ET function evaluated by sonotubometry

The duration of ET opening measured by sonotubometry in the EOM(+) group, EOM(-) group, and control group are shown in Fig. 1. The tubal opening duration in patients with ECRS which was not significantly different between the EOM (+) and EOM (-) groups was significantly longer than that in the

Table 2. Clinical characteristics of the three groups

	ECRS with bronchial asthma		Normal control
	EOM(+)	EOM(-)	
No. of patients (ear)	31(62)	16(32)	12(24)
M/F, No.	10/21	7/9	5/7
Age a	53.0±11.0 (32-75)	58.0±12.0 (29-72)	44.5±17.7 (30-77)
AIA	7	3	
CT score a	18.1±4.0 (4-30)	14.0±6.0(1-24)	
Eosinophils in PB,% a	7.0±5.2 (0-15.4)	10.4±5.2 (5-17.5)	
Serum IgE a	685.1±849.7 (13.6-4160)	367.9±268.5	
(20-921)			
Past history of FESS	19 (61.3%)	14 (87.5%)	
History of nasal irrigation	13	13	

ECRS, eosinophilic chronic rhinosinusitis; EOM, eosinophilic otitis media;
PB, peripheral blood; AIA, aspirin intolerance asthma;
FESS, functional endoscopic sinus surgery
a Mean ± standard deviation (range)

Table 3. Ages at onset of the diseases in patients

	EOM(+)	EOM(-)	P value
Bronchial asthma	34.1±11.9	43.0±13.0	P< 0.05
Rhinosinusitis	35.3±12.9	44.6±12.0	P< 0.05
EOM	46.3±11.9	-	

Table 4. Responses to the questionnaire for ear and nasal symptoms

	No. (% of respondents) ECRS with bronchial asthma		P value
Symptom	EOM(+)	EOM(-)	(χ2 test)
Rhinorrhea	27(87.1)	11(68.8)	0.13
Habitual sniffing	20(64.5)	4(25.0)	0.01*
Echo to an ambient sound	29(61.3)	2(12.5)	0.005**
Audition of loud breathing sounds	12(38.7)	2(12.5)	0.06
Autophony	17(54.8)	3(18.8)	0.02*
Feeling of ear stuffiness	23(74.2)	5(33.3)	0.003**

ECRS, eosinophilic chronic rhinosinusitis; EOM, eosinophilic otitis media
Statistically significant (*p<0.05, ** p<0.01)

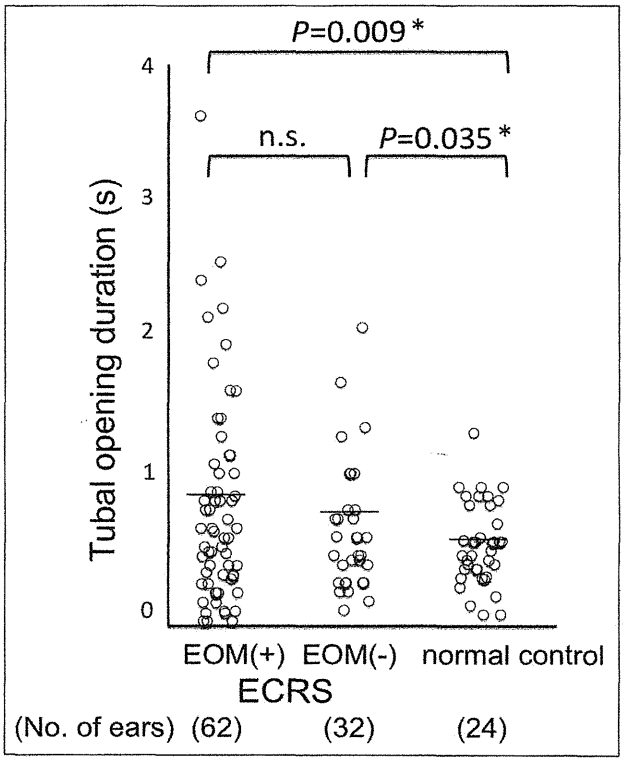

Figure 1. Tubal opening duration measured by sonotubometry in the three groups. ECRS, eosinophilic chronic rhinosinusitis; EOM, eosinophilic otitis media.

controls (p<0.05), suggesting that tubal closing may be insufficient in ECRS patients, regardless of the association with EOM. However, insufficiency of tubal closing appeared to be more severe in the EOM(+) group than in the EOM(-) group, as suggested by the smaller p value of the control group compared with the EOM(+) group than that of the control group compared with the EOM(-) group.

Discussion

EOM is recognized as an intractable otitis media and is characterized by the accumulation of eosinophils in middle ear effusion and middle ear mucosa. In 2011, diagnostic criteria were established[3], but the mechanism of accumulation of eosinophils in the middle ear has not been determined yet. A patulous ET detected by sonotubometry in patients with EOM was thought to be a possible factor for developing EOM because it might easily allow foreign materials to enter the middle ear and cause eosinophilic inflammation[4], but the cause of the patulous ET itself is unknown.

Sonotubometry is not routinely used to assess ET ventilatory function because its value for clinical practice and reproducibility has not been adequately demonstrated. However, the technique of sonotubometry has gradually improved and its results are currently at least as good as those of other function tests[12]. The occurrence of a false positive (no signal) in normal cases was considered as a problem, but this suggests that the ET does not necessarily open with each swallowing[13]. Electromyographic activity for paratubal muscles has been reported to be associated with peak ET opening evaluated by sonotubometry[14], and we believe that it is a simple and useful examination.

The sonotubometry of a patulous tube were classified into three types by Virtanen in 1978[15]: type I, a

relatively sharp peak of increase (normal); type II, well opens on swallowing and for some time afterwards, and closes little by little; and type III, remains open for a long time or is continuously open, and is closed by bending the head forward, by lying down, and (or) sniffing. We used the tubal opening duration to detect insufficient closing, which was mostly observed for type II.

In this study, the tubal opening duration in patients with ECRS and bronchial asthma as evaluated by sonotubometry was significantly longer than that in the controls. This finding suggests that closing of the ET may be insufficient in these patients, regardless of the association with EOM. Although the tubal opening duration was not significantly different between the EOM(+) and EOM(-) groups, the insufficiency of tubal closing appeared to be more severe in the EOM(+) group than in the EOM(-) group. Additional factors leading to EOM should be considered in the future.

ECRS patients with asthma who undergo functional endoscopic sinus surgery (FESS) have a worse postoperative condition than do chronic rhinosinusitis patients without asthma [5][16] and require aggressive medical management to prevent polyp recurrence, even after FESS. The standard medical therapy for ECRS patients is oral or topical corticosteroids and antileukotrienes [5]. Most of the patients in the present study had already received such medications for long periods of time as a treatment for asthma or ECRS. Some drugs may alter ventilatory function of the ET. Application of histamine solution around the nasopharyngeal ostium of ET-induced mucosal swelling has been observed to deteriorate ET function as measured by sonotubometry [17]. In patients with EOM, medications, such as corticosteroids and antileukotrienes, may reduce mucosal swelling around the ET, causing insufficiency of tubal closing.

Repeated nose blowing triggers the occurrence of EOM in patients who undergo FESS [7]. The most frequent episode in patients with EOM is the passing of air into the ear when blowing the nose [4]. An increase in nasopharyngeal pressure by blowing the nose might be one of the causes leading to EOM, particularly in the post-FESS state without polyposis. Therefore, patients with ECRS associated with asthma might have a risk for the onset of EOM because their ET function is unstable.

The effect of nasal irrigation is another possible factor that leads to EOM. Our questionnaire revealed that some patients had episodes of a feeling of ear fullness after nasal irrigation, and therefore, stopped doing it. Saline nasal irrigation itself is considered to be a safe treatment and is recommended for ECRS [18]. There are many types of douches, and some of them have difficulty in controlling water pressure. High-pressure irrigation might be a risk factor for the onset of EOM in the presence of insufficient tubal closing. This insufficiency allows antigenic materials to enter through the ET to the middle ear, causing eosinophilic-dominant inflammation. We recommend using a low-pressure douche and stopping nasal irrigation when the patient complains of aural symptoms.

This study clearly showed that most patients were affected by rhinosinusitis or bronchial asthma first, and otitis media developed approximately 10 years later. EOM may be the endpoint of eosinophilic inflammation involving the upper and lower airway tracts. In addition, the average age at onset of bronchial asthma in the EOM(+) group was almost 10 years earlier than that in the EOM(-) group. The early onset of adult-type asthma is also a risk of developing EOM. Further study is necessary to elucidate the onset of EOM.

Conclusion

Patients with both ECRS and bronchial asthma are at high risk of developing EOM. These patients should be carefully monitored regarding their ear symptoms, particularly when undergoing FESS and using nasal irrigation.

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Auris Nasus Larynx 40 (2013) 458–464

Contents lists available at SciVerse ScienceDirect

Auris Nasus Larynx

journal homepage: www.elsevier.com/locate/anl

Increased exhaled nitric oxide and its oxidation metabolism in eosinophilic chronic rhinosinusitis

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ARTICLE INFO

Article history:
Received 30 September 2012
Accepted 4 February 2013
Available online 13 March 2013

Keywords:
Chronic rhinosinusitis
Eosinophils
Interleukin-5
Nitric oxide
Nitric oxide synthase
Nitrotyrosine

ABSTRACT

Objective: Monitoring of fractional concentrations of exhaled nitric oxide (FeNO) has become a reliable marker of inflammation in human nose and paranasal sinuses. However, it is still unknown to what extent nasal NO levels contribute to the pathology of chronic rhinosinusitis (CRS). In the present study, we aimed to examine FeNO levels and the underlying mechanism of NO production and metabolism in patients with eosinophilic chronic rhinosinusitis (ECRS) and non-ECRS.

Methods: Thirty-three untreated ECRS patients, 16 non-ECRS patients, and 38 normal subjects were enrolled in this cross-sectional study of FeNO levels. Oral and nasal FeNO levels were measured before treatment using an electrochemical NO analyzer (NObreath®) with a nose adaptor. The mRNA expression of three nitric oxide synthase (NOS) isoforms, interleukin-5 (IL-5), and transforming growth factor-beta (TGF-β) in the ethmoid sinus mucosa and nasal polyps were analyzed by real-time PCR. Immunohistological localization of inducible NOS (iNOS) and nitrotyrosine (NT), a marker for oxidized NO metabolites, was also examined.

Results: ECRS patients showed significantly higher oral FeNO levels compared to non-ECRS patients and normal subjects (mean values, 47.6, 13.5, and 15.3 ppb, respectively). Nasal FeNO levels of the non-ECRS patients (30.5 ppb) were significantly lower than those of the ECRS patients (53.9 ppb) and normal subjects (45.5 ppb). Positive correlations existed between the blood eosinophil percentage and FeNO levels in ECRS patients. Histologically, ECRS patients showed higher eosinophil accumulation in the ethmoid mucosa than non-ECRS patients (103.1 vs. 16.3 cells/HPF). Real-time PCR analysis showed significant upregulation of iNOS and IL-5 mRNA expression in the ethmoid mucosa of the ECRS patients compared to those of non-ECRS patients. Positive iNOS immunoreactivity was observed in ciliated epithelial cells, submucosal glands and associated inflammatory cells in both groups. NT immunoreactivity was detected in the epithelium and around inflammatory cells. Intense NT staining was co-localized with eosinophil accumulation and ECRS patients showed significantly higher rates of NT-positive cells than non-ECRS patients.

Conclusion: A combination of oral and nasal FeNO measurement is a valid marker for the classification and definition of different CRS subtypes in Japan. Higher levels of oral and nasal FeNO in ECRS patients may reflect the persistence of eosinophilic inflammation in sinus mucosa with concomitant iNOS upregulation and accompanying deposition of oxidized NO metabolites.

1. Introduction

Nitric oxide (NO) has been proposed to have a variety of roles in the human nose and paranasal sinuses relevant to airway defense mechanisms, as well as being an inflammatory mediator [1,2]. The standardization of measuring techniques by the American Thoracic Society/European Respiratory Society has opened the way for the collection of comparable airway NO data in normal subjects and those with disease states [3,4]. Because the human paranasal sinuses are a major source of intrinsic NO production, the monitoring of fractional concentrations of exhaled NO (FeNO) in nasal airways can be a reliable marker of sinus inflammation [5–7]. Nasal NO levels are reported to be decreased in most patients with chronic rhinosinusitis (CRS); however, some contradictions still remain in the findings pertinent to classification of CRS types. In addition, it is still unclear to what extent nasal NO levels contribute to CRS pathology [8–10].

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http://dx.doi.org/10.1016/j.anl.2013.02.001