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ORIGINAL ARTICLE

Staphylococcus aureus nasal colonization increases the risk of cedar pollinosis

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Abstract

Background: One-third of the people in Japan are colonized with *Staphylococcus aureus* (*S. aureus*) and suffer from virulence factor-mediated subclinical inflammation of the nares. We investigated whether subclinical inflammation contributed to cedar pollinosis affecting 20 million people annually.

Methods: The study participants were 814 inhabitants of the A or B prefectures. We compared the colonization rate and population structure of *S. aureus*, in association with the prevalence of cedar pollinosis, between participants in these two areas.

Results: A prefecture had twice the annual amount of airborne cedar pollen compared with B. The prevalence of cedar pollinosis was significantly higher in A (23.5%) than in B (13.1%) ($p = 0.0004$). Moreover, the prevalence of cedar pollinosis was higher in female participants (23.3%) than in male participants (14.7%) ($p = 0.003$). In addition, the prevalence of cedar pollinosis was higher in S. aureus carriers (24.2%) than in S. aureus noncarriers (17.9%) ($p = 0.03$). The isolation rate of clonal complex (CC) 508 was higher in the A group (21%) than in the B group (7%) ($p = 0.015$).

Conclusion: Nasal colonization of *S. aureus* is a major risk factor for cedar pollinosis. However, the direct mechanism of this risk is currently unknown.

KEYWORDS
cedar pollinosis, clonal complex, MLST, *Staphylococcus aureus*

1 | INTRODUCTION

Cedar pollinosis is an IgE-mediated type I allergy with symptoms, such as allergic rhinitis, sinusitis, and rhinoconjunctivitis. Every pollen season in Japan, more than 20 million people suffer from this disease.[1–5]

Cumulative exposure to cedar pollen is the most important factor responsible for the induction of allergen-specific Th2-type responses.[1,6–8] However, even in cases where sensitization with Japanese cedar pollen is proven serologically, 50% of the people do not develop symptoms.[3] Conversely, staphylococcal superantigens, such as TSST-1 and SEB, augment IgE response in atopic patients who are exposed to allergens specific to atopy.[7,8] These observations suggest that staphylococcal superantigens may also augment the onset of cedar pollinosis in a person subclinically sensitized by cedar pollen. If this were the case, the colonization of *S. aureus* in more than one-third of Japanese people[9,10] would contribute to an increase in the prevalence of cedar pollinosis.

Staphylococcus aureus superantigens are the strongest T-cell activators and are obtained by horizontal gene transfer under the regulation of restriction modification systems.[11] SauI (type I) is one of the major systems that regulate the acquisition of exogenous virulence genes, and one of the constitutive genes responsible for target specificity, which is *hsdS*, perfectly correlates with clonal complex (CC). If superantigens are involved in cedar pollinosis, correlation with CC is also expected.

The accessory gene regulator (*agr*), quorum-sensing system, regulates the expression of staphylococcal superantigens.[12] The *agr* locus is comprised of a four-gene operon, *agrB*, *agrD*, *agrC*, *and agrA*, and polymorphisms centering on the former three loci (*agr* specificity group: referred to as *agr* subgroup type I through IV) influence quorum-sensing dynamics and result in variant expression of virulence genes in *S. aureus*.[12,13]

In the present study, first we epidemiologically investigated whether the colonization of *S. aureus* in the nares of inhabitants increases the prevalence of cedar pollinosis in the area. Then, we determined whether there was a correlation between *agr* type and cedar pollinosis and CC and cedar pollinosis.

2 | MATERIALS AND METHODS

2.1 | Study setting and design

Based on real-time airborne pollen data released by the Japanese Ministry of the Environment (http://kafun.taiki.go.jp), we selected two areas for our work: A and B prefectures. The former was an underpopulated area located on Shikoku Island, 89% of which consists of mountains. Most of the mountains (94%) are forested, and more than half of the forests (55%) are populated with Japanese cedar or, to a lesser extent, with another pollen producer, Japanese cypress. Contrarily, the total area occupied by Japanese cedar and cypress forests in B prefecture is equivalent to 7% of that in A prefecture. We conducted all of the studies in June and July, immediately following the end of the airborne pollen season of 2008.

2.2 | Participants and questionnaires

A total of 814 supermarkets' employees in both A (531 employees) and B (283 employees) prefectures participated in this study. We used written questionnaires, together with a verbal explanation of disease symptoms, to obtain data on gender, age, and history of cedar pollinosis (suffering from at least one of the following symptoms seasonally: runny nose, sneezing, blocked nose, itchy nose, itchy eyes, or watery eyes), and the duration of these symptoms. We include only current patients (people who have symptoms currently). Previous patients (people who do not have symptoms but had symptoms previously) were excluded.

2.3 | Isolation and identification of S. aureus

Nasal swabs were obtained from participants using wet (0.85% NaCl) cotton swabs, which were immediately inoculated on Staphylococcus Medium 110 (BD, Sparks, MD, USA) supplemented with 5% egg yolk (Kyokuto, Tokyo, Japan) and trypticase soy agar containing 5% sheep blood (Nippon BD, Tokyo, Japan). Plates were cultured for 48 h at 36°C. Identification of *S. aureus* was performed using API Staph (Sysmex-bioMérieux, Tokyo, Japan), and detection of *fem*A and B genes was performed using PCR.[11]

2.4 | Genotyping by MLST and agr subgroup type

We performed multilocus sequence typing (MLST) according to the method described by Enright et al.[14] Sequence types were determined by accessing the website for multilocus sequence typing (http://www.mlst.net/). We performed *agr* subgroup typing according to the method described by Francois et al., with minor modification.[15] We confirmed the *agr* subgroup type IV by using these specific primers: *agr*4f: 5′-AGCCTATTCCTGTGTGCGACTT-3′, and *agr*4r: 5′-ACGATAATGCCGTAATAATACCCGT-3′.

2.5 | Statistical analysis

We compared the prevalence of pollinosis in various situations employing chi-squared ($\chi 2$) test, Student's t-test, and multiple regression analysis. All statistical analyses were conducted using SPSS version 19.0 (Statistical Package for Social Science, Inc., Chicago, IL, USA).

2.6 | Ethical disclosure

The study was approved by the ethics committee of our institution, and informed consent to use the data for clinical investigation was obtained from the participants. The investigation was conducted in accordance with the Declaration of Helsinki of 1975.

3 | RESULTS

3.1 | Study participants

The participants of this study were 814 employees of 24 supermarkets in both the A (531) and B (283) prefectures. The mean participant age was 43.8 years in A and 42.8 years in B. The percentage of female participants was 69% in A and 46% in B prefectures.

3.2 | Levels of airborne cedar pollen

During the 2008 pollen season (February 2–May 18), 216,991 and 122,707 grains of airborne pollen (averages of data from two observation points in each area) were reported for A and B prefectures, respectively. The levels of airborne cedar pollen in A prefecture were almost double those in B.

3.3 | Risk factors for cedar pollinosis

Age was similar between participants with cedar pollinosis (42.8 years old) and without cedar pollinosis (43.4 years old) ($p = 0.54$) (Table 1). In contrast, the prevalence of cedar pollinosis was higher in female participants (23.3%) than in male participants (14.7%) ($p = 0.003$). Moreover, the prevalence of cedar pollinosis in A participants (23.5%) was significantly higher than that in B participants (13.1%) ($p = 0.0004$). In addition, the prevalence of cedar pollinosis was higher in *S. aureus* carriers (24.2%) than in *S. aureus* noncarriers (17.9%) ($p = 0.03$). Multiple regression analysis showed that gender, prefecture, and *S. aureus* colonization were independently increased the risk of cedar pollinosis ($p = 0.016$, 0.002, and 0.022, respectively).

3.4 | Rate of *S. aureus* colonization

The rate of *S. aureus* colonization in A female, B female, A male, and B male was 29.3%, 35.3%, 32.5%, and 34.4% (average: 31.9%), respectively, and a statistically significant difference was not observed between these values. The *S. aureus* isolation rate from pollinosis participants was 38.4% in A and 40.5% in B ($p = 0.815$).

TABLE 1 Relationship between age, gender, prefectures, *Staphylococcus aureus* colonization and cedar pollinosis

	Cedar pollinosis		*p* value	
	(+)	(−)	$\chi 2$ test/t-test	multiple regression test
Age	42.8	43.4	0.54	0.370
Gender				
Male	47 (14.7%)	273		
Female	115 (23.3%)	379	0.003	0.016
Prefectures				
A	125 (23.5%)	406		
B	37 (13.1%)	246	0.0004	0.002
S. aureus colonization				
+	63 (24.2%)	197		
−	99 (17.9%)	455	0.03	0.022

3.5 | Association between the population structure of S. aureus and the prevalence of cedar pollinosis

Table 2 presents the relationship between CC and prevalence of cedar pollinosis. There were particularly high and low prevalence depending on CC in participants, such as CC 30 (43%) and CC 5 (0%) ($p = 0.005$), but these were not statistically significant overall, and CC did not increase the incidence of cedar pollinosis. Table 3 presents the relationship between *agr* type and prevalence of cedar pollinosis. None of the *agr* types increased the incidence of cedar pollinosis ($p = 0.963$).

3.6 | Association between population structure of *S. aureus* and area

Table 4 presents the population structures of 208 *S. aureus* strains. We found genotypic diversity of *S. aureus* in both areas with more than 18 CCs observed. Except for CC508, which had a higher isolation rate in A (21%) than in B (7%) ($p = 0.015$), no significant difference was observed in the composition of CCs between A and B prefectures. We observed no significant difference in the composition of *agr* types between A and B prefectures (data not shown).

4 | DISCUSSION

In this study, we investigated the prevalence of cedar pollinosis in two areas. Living in the A prefecture was a major risk of cedar

TABLE 2 Clonal complexes (CCs) of *Staphylococcus aureus* and prevalence of cedar pollinosis

CCs	Prevalence of cedar pollinosis (%)	*p* value (compared to CC30)
CC188	18	
CC1	25	
CC101/96	0	0.067
CC7, 6	40	
CC5	0	0.005
CC97	20	
CC8	27	
CC20	33	
CC15	24	
CC12	24	
CC25/59/509	8	
CC121	18	
CC30	43	
CC508	32	
Singleton	29	

TABLE 3 *agr* type of *Staphylococcus aureus* and prevalence of cedar pollinosis

	Agr type				
Cedar pollinosis	Type I	Type II	Type III	Type IV	Total
+	31 (23%)	12 (20%)	1 (25%)	2 (20%)	46 (22%)
−	103	48	3	8	162
Total	134	60	4	10	208
p value (χ2 test)					0.963

TABLE 4 Clonal complexes (CCs) of *Staphylococcus aureus* isolated from A and B prefectures

	Prefectures		
CCs	A	B	*p* value
CC188	18	15	
CC1	7	1	
CC101 / 96	5	1	
CC7, 6	6	4	
CC5	10	5	
CC97	5	0	
CC8	9	6	
CC20	5	4	
CC15	13	12	
CC12	10	7	
CC25 / 59 / 509	8	5	
CC121	7	4	
CC30	4	3	
CC508	28	5	0.015
Singleton	1	0	
Total	136	72	

pollinosis, compared to those in the B area. The prevalence of cedar pollinosis in the research area was proportional to the amount of cedar pollen.

More than half of the people sensitized serologically by cedar pollen do not develop cedar pollinosis.[3] Some additional stimulation, such as atrophic nasal change caused by hormonal imbalance in perimenopausal women, may contribute to cedar pollinosis progression.[16] In fact, pollinosis was more frequently found in women than men ($p = 0.03$). Subclinical nasal inflammation caused by *S. aureus* colonization may contribute to cedar pollinosis progression.[11,17,18] Here, we have reported that colonization of *S. aureus* in the nares is a risk factor for cedar pollinosis ($p = 0.03$). We hypothesized that the rates of *S. aureus* colonization would be higher in A participants or female participants; however, the rates were not significantly different.

We analyzed the association between the population structure of *S. aureus* and the prevalence of cedar pollinosis. We found no difference in the strength of risk among CCs. It is postulated that among these diverse *S. aureus* lineages, certain clones, such as CC 30, found in *S. aureus* carriers are more likely to cause invasive diseases than others.[19,20] However, the isolation rate of CC 30 in the Japanese community is very low, and CC 30 is not considered an infectious threat in Japanese communities. Thus, *S. aureus* lineages that actually pose an infectious or allergic threat have not yet been identified.

Staphylococcus aureus superantigens participate in allergic disease development,[7,8] so we predicted that *agr* type can reflect the strength of each *S. aureus* interaction with cedar pollinosis. Investigators have reported that there are predilections of *agr* phenotypes for *S. aureus* pathotypes.[21,22] According to a report by Geisinger et al., these phenomena are caused by the variant expression of virulence genes due to polymorphisms within *agrBDC* genes in a QC system.[13] However, contrary to our expectations, the present data indicated that all *agr* strains have approximately the same frequency of cedar pollinosis. We did not observe an *agr* type that exhibited a special correlation with cedar pollinosis. Taken together, we did not observe different levels of risk between the strains.

We analyzed the association between the population structure of S. aureus and area. The population structure of *S. aureus* is extremely diverse,[23–25] and CC profiles exhibit a geographical feature. Regions with high isolation rates of CC 30, CC 45, and CC 8 are Algeria, Moldova, and France. Cambodia and Chengdu have higher CC 121 and lower CC 8. In Mali, CC 15 and CC 152 are higher. Unlike these regions, CC 188 and CC 508 were specifically high in Japan in the present study. However, we did not observe any difference in the population structures of *S. aureus* between the investigation areas.

We identified several limitations in the present study. First, we selected the supermarket employees as the subject of samples. Therefore, our study cohort included only those aged <62 years. Because immune responses are different among different age groups, the results may not be generalizable because of the possibility of sampling bias. Second, the clinical diagnosis of cedar pollinosis was done by a written questionnaire, not a doctor visit. Therefore, it may be difficult to separate cedar pollinosis and other diseases. Third, this study was conducted in two areas in Japan. Therefore, it might be difficult to generalize the status of *S. aureus* circulation in Japan from these results. Finally, the discriminatory power of MLST may not be sufficient to determine the diversity of *S. aureus*. Inclusion of multiplex PCR-based SCCmec typing would increase the relevance of the data.

Nasal colonization of *S. aureus* is certainly a risk factor for cedar pollinosis. However, the direct mechanism of this risk is currently unknown. We have to analyze the association between the population

structure of S. aureus and the prevalence of cedar pollinosis in more areas. Our goal is to identify S. aureus lineages that actually pose an allergic threat.

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CONFLICT OF INTEREST

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

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REFERENCES

1. Okuda M. Epidemiology of Japanese cedar pollinosis throughout Japan. Ann Allergy Asthma Immunol. 2003;91:288–96.
2. Kaneko Y, Motohashi Y, Nakamura H, Endo T, Eboshida A. Increasing prevalence of Japanese cedar pollinosis: a meta-regression analysis. Int Arch Allergy Immunol. 2005;136:365–71.
3. Ozasa K, Hama T, Dejima K, Watanabe Y, Hyo S, Terada T, *et al*. A 13-year study of Japanese cedar pollinosis in Japanese schoolchildren. Allergol Int. 2008;57:175–80.
4. Panzani R, Yasueda H, Shimizu T, Shida T. Cross-reactivity between the pollens of Cupressus sempervirens (common cypress) and of Cryptomeria japonica (Japanese cedar). Ann Allergy. 1986;57:26–30.
5. Hama T, Miyazaki M, Dejima K, Hisa Y, Fujieda S, Saito H, *et al*. Clinical study of the paranasal sinuses of patients with Japanese Cedar pollinosis. Nihon Jibiinkoka Gakkai Kaiho. 2002;105:1078–86.
6. Takasaki K, Enatsu K, Kumagami H, Takahashi H. Relationship between airborne pollen count and treatment outcome in Japanese cedar pollinosis patients. Eur Arch Otorhinolaryngol. 2009;266:673–6.
7. Yamanishi T, Chikamatsu K, Takahashi G, Endo S, Masuyama K. Immune regulation byCD4+CD25+ regulatory T calls in patients with Japanese cedar pollinosis. Int Arch Allergy Immunol. 2011;156:187–95.
8. Hofer MF, Harbeck RJ, Schlievert PM, Leung DYM. Staphylococcal toxins augment specific IgE responses by atopic patients exposed to allergen. J Invest Dermatol. 1999;112:171–6.
9. Shiomori T, Yoshida S, Miyamoto H, Makishima K. Relationship of nasal carriage of *Staphylococcus aureus* to pathogenesis of perennial allergic rhinitis. J Allergy Clin Immunol. 2000;105:449–54.
10. Gorwitz RJ, Kruszon-Moran D, McAllister SK, McQuillan G, McDougal LK, Fosheim GE, *et al*. Changes in the prevalence of nasal colonization with Staphylococcus aureus in the United States, 2001–2004. J Infect Dis. 2008;197:1226–34.
11. Omoe K, Hu DL, Takahashi-Omoe H, Nakane A, Shinagawa K. Comprehensive analysis of classical and newly described staphylococcal superantigenic toxin genes in *Staphylococcus aureus* isolates. FEMS Microbiol Lett. 2005;246:191–8.
12. Novick RP, Geisinger E. Quorum sensing in staphylococci. Annu Rev Genet. 2008;42:541–64.
13. Geisinger E, Chen J, Novick RP. Allele-dependent differences in quorum-sensing dynamics result in variant expression of virulence genes in Staphylococcus aureus. J Bacteriol. 2012;194:2854–64.
14. Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of Staphylococcus aureus. J Clin Microbiol. 2000;38:1008–15.
15. Francois P, Koessler T, Huyghe A, Harbarth S, Bento M, Lew D, *et al*. Rapid Staphylococcus aureus agr type determination by a novel multiplex real-time quantitative PCR assay. J Clin Microbiol. 2006;44:1892–5.
16. Bousquet J, Khaltaev N, Cruz AA, Denburg J, Fokkens WJ, Togias A, *et al*. Allergic Rhinitis and its Impact on Asthma (ARIA) 2008. Allergy. 2008;63:8–160.
17. Cole AM, Tahk S, Oren A, Yoshioka D, Kim YH, Park A, *et al*. Determinants of Staphylococcus aureus nasal carriage. Clin Diagn Lab Immunol. 2001;8:1064–9.
18. O'Brien GJ, Riddell G, Elborn JS, Ennis M, Skibinski G. Staphylococcus aureus enterotoxins induce IL-8 secretion by human nasal epithelial cells. Respir Res. 2006;7:115.
19. Wertheim HF, van Leeuwen WB, Snijders S, Vos MC, Voss A, Vandenbroucke-Grauls CM, *et al*. Associations between *Staphylococcus aureus* genotype, infection, and in-hospital mortality: a nested case-control study. J Infect Dis. 2005;192:1196–200.
20. Melles DC, Gorkink RFJ, Boelens HAM, Snijders SV, Peeters JK, Moorhouse MJ, *et al*. Natural population dynamics and expansionof pathogenic clones of *Staphylococcus aureus*. J Clin Invest. 2004;114:1732–40.
21. Jarraud S, Mougel C, Thioulouse J, Lina G, Meugnier H, Forey F, *et al*. Relationships between *Staphylococcus aureus* genetic background, virulence factors, agrgroups (alleles), and human disease. Infect Immun. 2002;70:631–41.
22. Tristan A, Bes M, Meugnier H, Lina G, Bozdogan B, Courvalin P, *et al*. Global distribution of Panton-Valentine leukocidin-positive methicillin-resistant *Staphylococcus aureus*, 2006. Emerg Infect Dis. 2007;13:594–600.
23. Ruimy R, Armand-Lefevre L, Barbier F, Ruppé E, Cocojaru R, Mesli Y, *et al*. Comparisons between Geographically diverse samples of carried *Staphylococcus aureus*. J Bacteriol. 2009;191:5577–83.
24. Ruimy R, Maiga A, Armand-Lefevre L, Maiga I, Diallo A, Koumaré AK, *et al*. The carriage population of *Staphylococcus aureus* from Mali is composed of a combination of pandemic clones and the divergent Panton-Valentine Leukocidin-positive genotype ST152. J Bacteriol. 2008;190:3962–8.
25. Fan J, Shu M, Zhang GE, Zhou W, Jiang Y, Zhu YU, *et al*. Biogeography and virulence of *Staphylococcus aureus*. PLoS One. 2009;4:e6216.

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