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.\textsuperscript{1-5}  

Cumulative exposure to cedar pollen is the most important factor responsible for the induction of allergen-specific Th2-type responses.\textsuperscript{1,6-8}  

However, even in cases where sensitization with Japanese cedar pollen is proven serologically, 50% of the people do not develop symptoms.\textsuperscript{3}  

Conversely, staphylococcal superantigens, such as TSST-1 and SEB, augment IgE response in atopic patients who are exposed to allergens specific to atopy.\textsuperscript{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 \textit{S. aureus} in more than one-third of Japanese people\textsuperscript{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. Saul (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 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. 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.

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 femA and B genes was performed using PCR.

2.4 | Genotyping by MLST and agr subgroup type

We performed multilocus sequence typing (MLST) according to the method described by Enright et al. 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. We confirmed the agr subgroup type IV by using these specific primers: agr4f: 5′-AGGCTATTCTCTGTGTGCGACTT-3′, and agr4r: 5′-ACGATAATGCCTAATAATACCCGT-3′.

2.5 | Statistical analysis

We compared the prevalence of pollinosis in various situations employing chi-squared (χ²) 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     | 0.0004              | 0.002                   |
| B                        | 37 (13.1%)       | 246     |                     |                         |
| S. aureus colonization   |                  |         |                     |                         |
| +                        | 63 (24.2%)       | 197     | 0.03                | 0.022                   |
| -                        | 99 (17.9%)       | 455     |                     |                         |

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 CC30 (43%) and CC5 (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 (31%) than in B (155) ($p = 0.015$), 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
pollinosis. Some additional stimuli, 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 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

### Table 3

| 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|         |

### Table 4

| Ceramic pollinosis | Agr type | Total |
|--------------------|----------|-------|
|                     | Type I   | Type II | Type III | Type IV |
| +                   | 31 (23%) | 12 (20%) | 1 (25%)  | 2 (20%)  |
| -                   | 103      | 48      | 3        | 8        |
| Total               | 134      | 60      | 4        | 10       |

p value (χ² test) 0.963
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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