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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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The presence of virus significantly associates with chronic rhinosinusitis disease severity

To the Editor,

Chronic rhinosinusitis (CRS) is an inflammatory disorder of the paranasal sinuses occurring with and without nasal polyps (CRSsNP and CRSwNP). Although not objectively demonstrated, an initial viral insult is commonly described by patients prior to the development of CRS. If viruses were demonstrated to play a role in CRS, novel prophylactic and/or therapeutic targets might be uncovered.

Findings in previous studies investigating CRS and viruses are variable.[1-4] Possible reasons include small sample sizes, unvalidated collection methods, seasonal limitation, heterogenous CRS cohorts, and limited viral species screening. No studies to date have investigated disease severity in relation to viral presence.

We aimed to investigate the sinonasal virome of patients with CRS in relation to disease phenotype, to compare it to healthy controls, and to explore any association between more severe disease and viral presence. Cytobrush samples were taken from the sinonasal passages, and DNA/RNA extracts underwent PCR for a number of viral species and strains. The *Herpesviridae* were excluded due to their near-ubiquity in adult sinuses. Methodology details appear in the Data S1.

A total of 288 patients were recruited: 71 controls, 133 CRSsNP, and 84 CRSwNP (Table S1). Of the 288, 45 patients were virus-positive: 5 control, 27 CRSsNP, and 13 CRSwNP (Figure 1). The rate of viral positivity was significantly higher in the CRSsNP group ($P < 0.05$).

Objective disease severity scores (Lund-Mackay [LMS] and Lund-Kennedy [LKS]) revealed significantly worse disease in the CRSsNP virus-positive cohort compared with the CRSsNP virus-negative cohort ($P < 0.05$, Figure 2). No significant differences were observed in the control or CRSwNP cohorts. Subjective scores (Sino-Nasal Outcome Test 22 [SNOT-22] and Adelaide Disease Severity Score [ADSS]) revealed no difference between patients with or without virus in any of the groups (Figure 2). PCR cycle thresholds also revealed no difference between virus-positive or virus-negative individuals (Table S2). Viral species detected did not vary significantly from the previously published studies; these were largely rhinovirus and coronavirus (Tables S2 and S3 and Figure S1). Peak viral detection occurred in spring and winter; there was no significant difference in detection when analyzed by season (Figure S2).

This study identified common respiratory viruses as more prevalent in patients with CRSsNP than in controls. It is the first study to demonstrate their significant association with more severe radiological and endoscopic disease in *virus-positive* CRSsNP patients but not *virus-positive* patients with CRSwNP.

The lack of any significant difference in subjective symptom scores in any of the groups is not unexpected. The absence of correlation between subjective and objective measures of disease severity has been well documented.[5] Although the inclusion of nonrhinologic questions in the SNOT-22 score is a possible explanation, no difference was observed when using the more specific ADSS. Another possible explanation may be the timing of sampling. As most viruses tested in the assay are shed from the nasopharynx up to 3 weeks after symptom resolution, it is possible that sampling occurred either during this time or early in the infection prior to symptom development.

The viruses identified largely were consistent with those seen in previous CRS studies, with the exception that this study did not identify metapneumovirus. The main viruses observed across all cohorts were rhinovirus and coronavirus, with influenza featuring strongly in the CRS group. However, it seems likely there is no one

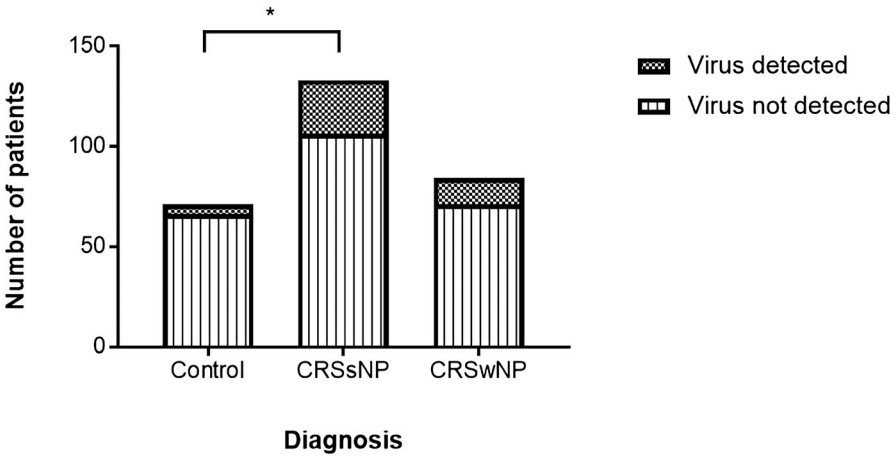

FIGURE 1 Detection of virus by PCR in controls and in patients with chronic rhinosinusitis (CRS). Number of patients where virus was detected and not detected in controls, CRSsNP and CRSwNP. Comparison of positivity of virus detected, *$P < 0.05$, chi-square test

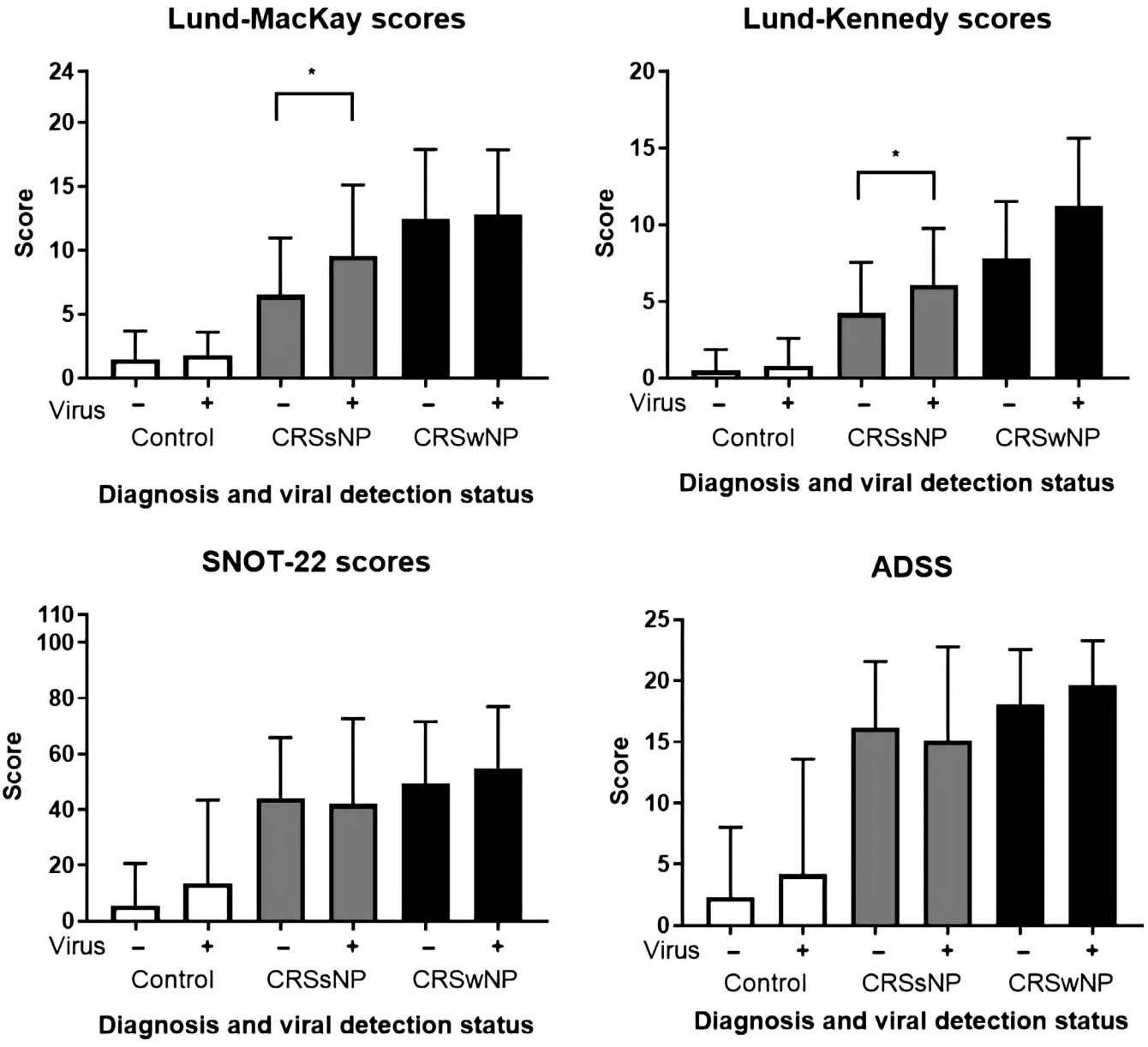

FIGURE 2 Objective (top) and subjective (bottom) disease scores in virus-negative and virus-positive patients. Absolute improvement units grouped and compared by diagnosis (control, CRSsNP, or CRSwNP), * $P < 0.05$, Kruskal-Wallis tests

virus with a particular contribution to CRS not also seen in the general population. The size of the virus-positive cohort limited subanalysis of viral species/strains with regard to disease severity. As such, we were unable to determine whether one particular viral species is associated with worse disease.

Seasonality is an important concern when sampling for respiratory viruses. These are known to be most prevalent in winter and the early part of spring, an observation supported by this study. Importantly, we also showed strong viral positivity in summer and autumn. This highlights a clear shortfall of previous CRS virome studies that limited sampling to winter and spring.

The mechanisms underlying the viral contribution to CRS are unknown. Similarly unknown is whether the higher rates of viral infection here observed are a cause or a consequence of CRS. CRS has been well established as a bacterial disease, encompassing bacterial overgrowth, superantigen and biofilm formation, and disruption of the microbiome.[6] The link between the bacterial and viral hypotheses of CRS aetiopathogenesis may lie in the ability of viruses to prime the airways for bacterial infection. Viruses damage the epithelial barrier by increasing mucus production, reducing ciliary presence, and reducing tight junction expression. Viruses bind directly to bacteria and upregulate host cell surface molecules to facilitate bacterial-host adherence. Viruses hamper the innate immune system with effects on neutrophil and macrophage recruitment and impairment of natural killer, antigen presenting and T cell activity, leaving the mucosa at risk for bacterial invasion. Viruses alter temperature and variably exhaust or increase the availability of micronutrients, which can allow bacteria in planktonic and biofilm form to proliferate (References in Data S1). The general population, however, is subject to a near-constant onslaught from respiratory viruses but only 16% of individuals develop CRS. The link between these two entities may be related to the interferons (IFN) and their signaling pathways. These cytokines are expressed by almost all cells in response to pathogen invasion and play key roles in innate antiviral immunity. Deficient IFN responses to viral infection have been consistently demonstrated in asthma, a similar disease process to CRS, as well as in CRS itself.[7-9] We hypothesize this lack of early antiviral activity in patients with CRS may result in more frequent, severe, or persistent viral infections, paving the way for the bacterial invasion so critical for disease development. Further research, however, is required to clarify this hypothesis.

Our results confirm a long-held suspicion that viruses are more common in CRS than in the general population. Viral presence is associated with more severe sinus disease measured by LMS and LKS. This has the potential to lead to exciting new developments in viral prophylaxis and antiviral therapy in the prevention and possible treatment of CRS.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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SUPPORTING INFORMATION

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The change in the prevalence of wheeze, eczema and rhino-conjunctivitis among Japanese children: Findings from 3 nationwide cross-sectional surveys between 2005 and 2015

To the Editor,

It has been suggested that the global increase in prevalence of allergic disease since the 1980s, also observed in Japan, may have reached a plateau or even decreased in some areas.[1] The analysis of time trends in the prevalence requires the use of the same methodology at each time point. We have carried out a nationwide study in 2005, 2008 and 2015 using a validated questionnaire and a similar sampling method at each time point to investigate the changing prevalence of allergic disease among Japanese children.

The method of this study has been described in detail elsewhere.[2] Briefly, in 2005, 2008 and 2015, we conducted a school-based questionnaire survey among two age groups: students in years 1-2 (age 6-8 years old) and in years 8-9 (age 13-15 years old). Schools were randomly selected to have a total of 1000 students for both age groups from each and all 47 prefectures in Japan. Questionnaires with an information sheet for parents were distributed through schools, and students returning their questionnaires to schools were judged as giving consent to participate. We used the Japanese version of ISAAC (International Study of Asthma and Allergies in Childhood) core questions for asthma, allergic rhinitis and eczema to identify children with current and severe wheeze, rhino-conjunctivitis and eczema[3] (Table S1). Responses for age, sex and status of either current wheeze, current rhino-conjunctivitis or current eczema completed were included for analysis. The prevalence estimate for each allergic disease was calculated as the observed proportion with 95% confidence interval (CI) for each age group. Generalized linear model was employed to assess the change in the prevalence in each 2008 and 2015 by estimating a relative risk ratio (RR) using 2005 as baseline category. All statistical analysis was undertaken using Stata version 15.1 (StataCorp, College Station, TX, USA). The study protocol was approved by the Institutional Review Board of Tokyo Metropolitan Children's Medical Center (ID: H26-123).

Table S2 describes the participation for each study. The response rate was over 70% among both age groups for all 3 years. Over the 3 years, a total of 138 017 children aged 6-8 years old and 134 402 children aged 13-15 years old were included in the analysis.

As described in Table 1, the prevalence of current wheeze among 6- to 8-year-olds was stable between 2005 and 2008 (13.6% in both years), followed by a decrease to 10.2% in 2015 (RR 0.75, 95% CI 0.72-0.77). A similar but weaker trend was found for 13- to 15-year-olds between 2005 and 2015 (8.7% and 8.2%, respectively, RR 0.94, 95% CI 0.90-0.98). Severe wheeze almost halved in the 10 years for 6- to 8-year-olds (4.8%-2.9%, RR 0.60, 95% CI 0.56-0.64) and showed a slight decrease for 13- to 15-year-olds (3.6%-3.1%, RR 0.85, 95% CI 0.79-0.92). Rhino-conjunctivitis increased continuously for both age groups between the three study years, 14.8% in 2005 to 18.7% in 2015 (RR 1.27, 95% CI 1.23-1.30) for 6- to 8-year-olds and 20.5%-26.7% among 13- to 15-year-olds (RR 1.30, 95% CI 1.27-1.33). Severe rhino-conjunctivitis showed an increase only in 13- to 15-year-olds (5.8%-7.1%, RR 1.21, 95% CI 1.15-1.27). Current eczema decreased slightly in 6- to 8-year-olds (15.8%-14.6%, RR 0.92, 95% CI 0.89-0.95) and was stable for 13- to 15-year-olds between 2005 and 2015 (9.9%-9.7%, RR 0.98, 95% CI 0.94-1.02), while there was a slight decrease in severe eczema among 13- to 15-year-olds (1.9%-1.6%, RR 0.83, 95% CI, 0.74-0.92) but no change among 6- to 8-year-olds.

The overlap of the three allergic diseases among the two age groups in 2005 and 2015 is described in Figure 1, by means of proportional Venn diagrams. Among 6- to 8-year-olds, although the prevalence of children with wheeze, eczema or both decreased between 2005 and 2015, the overall proportion with any allergic disease was similar (33.7% and 34.7%), due to the increase in children with rhino-conjunctivitis only (8.5%-12.5%). Similarly, among 13- to 15-year-olds, the increase in rhino-conjunctivitis only (15.3%-20.3%) or with comorbid wheeze or eczema (5.2%-6.5%) resulted in an overall increase in children with any allergic disease (31.9%-36.5%).

Our results suggest that the prevalence of wheeze and eczema has stabilized or declined in Japanese children. It has been speculated that the plateau in prevalence is showing that the saturation level, determined by the genetic composition of the population, has been achieved.[4] However, we found that the prevalence of rhino-conjunctivitis was still rising, in