

## Respiratory Viral Infection in the Chronic Persistent Phase of Chronic Rhinosinusitis

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**Objectives/Hypothesis:** The role of respiratory viral infection in the pathogenesis of chronic rhinosinusitis (CRS) has been rarely studied and remains controversial. The aim of this study was to explore the prevalence of respiratory viruses in the chronic status of CRS.

**Study Design:** A case-control prospective study.

**Methods:** Fifty-three control subjects, and 67 CRS with nasal polyp (CRSwNP) and 61 CRS without nasal polyp (CRSsNP) patients without signs of acute viral infection were enrolled. Epithelial cells scraped from the middle nasal meatus were tested for the nucleic acid of nine common respiratory viruses using polymerase chain reaction assay. The clinical disease severity was compared between subjects with and without viral infection.

**Results:** The overall detection rate of viral infection was 75.47%, 68.66%, and 73.77% in controls, CRSwNP, and CRSsNP, respectively, and no significant difference among studied groups was observed. There was no significant difference in detection rate of any specific individual virus or multiple viruses among the groups studied either. Visual analog scale scores of symptoms, computed tomography scores, or endoscope scores did not show obvious difference between subjects with and without viral infection.

**Conclusions:** Although a high frequency of viral infection could be observed in the middle nasal meatus, no increase of frequency of viral infection could be demonstrated in chronic persistent phase of CRSsNP and CRSwNP. The contribution of the interaction between viral infection and host immunity to the pathogenesis of CRS remains to be determined.

**Key Words:** Chronic rhinosinusitis, infection, respiratory virus.

**Level of Evidence:** 3b.

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### INTRODUCTION

Chronic rhinosinusitis (CRS) is characterized by inflammation of the mucosa of the nose and paranasal sinuses. It remains a major public health problem often associated with poor outcomes after standard medical therapy and endoscopic sinus surgery.<sup>1,2</sup> Primarily based on the absence or presence of nasal polyps, CRS is currently divided into two types, CRS without nasal polyps (CRSsNP) and CRS with nasal polyps (CRSwNP).<sup>1,2</sup> CRSsNP and CRSwNP are characterized by distinctive expression patterns of inflammatory and remodeling

mediators, suggesting that they may have different etiologies.<sup>1,2</sup> During the last decade, although significant progress has been made regarding the immunopathologic characteristics of CRS, the initial triggers of the persistent inflammation in CRS remain unknown. In some specific subgroups of CRS patients, bacterial or fungal infection has been suggested to be a trigger and/or modifier of the inflammation process in CRS.<sup>1,2</sup> Respiratory virus planting is a common condition in the upper respiratory tract, and acute rhinosinusitis is thought to be initiated by a respiratory virus infection.<sup>3,4</sup> In vitro studies have demonstrated that respiratory viral infection can cause damage to nasal epithelial cells, impair tight junctions in nasal epithelial cells, and facilitate invasion of bacteria into nasal mucosa.<sup>5,6</sup> Nevertheless, few studies have explored the association between viral infection and CRS, and the prevalence of a viral pathogen in CRS patients remain uncertain.<sup>7–9</sup> Recently, Cho et al. detected a higher prevalence of respiratory viruses, particularly rhinovirus, in nasal lavage fluid and inferior turbinate epithelial cell samples from CRS patients than that seen in controls, suggesting an association between respiratory viral infection and CRS. However, a significant limitation of Cho et al.'s study is that nasal lavage fluid samples and epithelial cell samples scraped from inferior turbinate may be unable to reflect the condition in the sinuses and middle meatus, where the development of CRS takes place.<sup>7</sup> In another study, Wood et al. investigated the presence of a respiratory virus in the

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TABLE I.  
Patients' Clinical Data.

	Control	CRSwNP	CRSsNP	Control vs. CRSwNP, P Value	Control vs. CRSsNP, P Value	CRSsNP vs. CRSwNP, P Value
No. of patients	53	67	61	—	—	—
Male, no. (%)	42 (79.25)	45 (67.16)	44 (72.13)	.141	.379	.542
Age, yr, median (IQR)	27 (20–43.5)	27 (21–41)	28 (19–39)	.853	.537	.459
Patients with allergic rhinitis, no. (%)	8 (15.09)	12 (17.91)	15 (24.59)	.681	.208	.355
Patients with asthma, no. (%)	2 (3.77)	4 (5.97)	5 (8.20)	.693	.447	.736
Total symptoms scores, median (IQR)	8 (5–13)	18 (15–24)	17 (13.5–23.5)	<.001	<.001	.602
CT scores, median (IQR)	0 (0–1)	13 (9–16)	10 (7–13)	<.001	<.001	.006
Endoscopy scores, median (IQR)	2 (0–3)	7 (4–11)	4 (3–5.5)	<.001	<.001	<.001

CT = computed tomography; CRSsNP = chronic rhinosinusitis without nasal polyps; CRSwNP = chronic rhinosinusitis with nasal polyps; IQR = interquartile range.

sinus mucosa from CRS patients and controls and did not detect any respiratory virus in all the samples examined, disfavoring a role of viral infection in CRS. Unfortunately, the very limited sample size (five CRSwNP patients, eight CRSsNP patients, and two controls) in Wood et al.'s study might lead to a biased result.<sup>8</sup> Our preliminary study on four common respiratory viruses failed to find any difference in respiratory virus detection rate in nasal epithelial cells scraped from the middle meatus between controls and CRS patients, although a high frequency of virus presence was demonstrated in samples from both groups.<sup>10</sup> In this prospective study, we extended our previous preliminary study and tested the nucleic acid of nine common respiratory viruses in epithelial cells

scraped from the middle meatus in a comparatively larger population. Moreover, the relationship between viral infection and clinical disease severity was evaluated. We found that there was no difference in virus detection rate among controls, CRSsNP patients, and CRSwNP patients, and there was no association between viral infection and clinical disease severity.

## MATERIALS AND METHODS

### Subjects and Evaluation

This study was approved by the ethics committee of Tongji Hospital of Huazhong University of Science and Technology, Wuhan, China, and was conducted with written informed

TABLE II.  
Primers Used for Reverse Transcription–Polymerase Chain Reaction Detection of Viruses.

Primer	Sequences	Expected Product Size (bp)	Annealing Temperature (°C)
Picornavirus	(F) 5'-CGGACACCCAAAGTAG-3'	389	57
	(R) 5'-GCACTTCTGTTTCCCC-3'		
Respiratory syncytial virus	(F) 5'-GCGATGTCTAGGTTAGGAAGAA-3'	409	53
	(R) 5'-GCTATGTCCTTGGGTAGTAAGCCT-3'		
Influenza virus type A	(F) 5'-AAGGGCTTTCACCGAAGAGG-3'	189	52
	(R) 5'-CCCATTCTCATTACTGCTTC-3'		
Influenza virus type B	(F) 5'-ATGGCCATCGGATCCTCAAC-3'	240	57
	(R) 5'-TGTCAGCTATTATGGAGCTG-3'		
Parainfluenza virus 1	(F) 5'-CTTCTGTCTGATTAATTCTG-3'	450	57
	(R) 5'-AGGATACATATCTGAATTTAAG-3'		
Parainfluenza virus 2	(F) 5'-CAGCAGATTGTTGTATTATCC-3'	400	57
	(R) 5'-CAAAACATCCCAACATAACCTGTGCCGGTA-3'		
Parainfluenza virus 3	(F) 5'-CTCGAGGTTGTCAGGATATAG-3'	500	57
	(R) 5'-CTTGGGAGTTGAACACAGTT-3'		
Human coronavirus OC43	(F) 5'-AGGAAGGTCTGCTCCTAATTCC-3'	300	53
	(R) 5'-TGCAAAGATGGGGAACTGTGGG-3'		
Human coronavirus 229E	(F) 5'-GGTACTCCTAAGCCTTCTCG-3'	370	53
	(R) 5'-TGCACTAGGGTTAAGAAGAGG-3'		
GAPDH	(F) 5'-GGAAGATGGTGTGGGATT-3'	265	60
	(R) 5'-GAAGGTGAAGTCCGGAGTC-3'		

GAPDH = glyceraldehyde phosphate dehydrogenase.

consent from all patients. A total of 67 CRSwNP and 61 CRSsNP patients were recruited. CRSwNP and CRSsNP were diagnosed according to the current US guidelines and the European Position Paper on Rhinosinusitis and Nasal Polyps.<sup>1,2</sup> All recruited patients suffered from bilateral CRS with invasion of at least the maxillary and ethmoidal sinus. Fifty-three patients undergoing septoplasty because of anatomic variations and not having sinus disease were enrolled as control subjects. All participants were enrolled from March 2010 to January 2013. The sample size was approximately equally distributed over the study period. None of the patients had an acute upper respiratory infection during the 4 weeks before the recruitment or a history of aspirin sensitivity. All patients stopped oral and topic application of corticosteroids at least 3 months and 1 month before the study, respectively. Patients were excluded if they had a diagnosis of cystic fibrosis, antrochoanal polyps, congenital mucociliary problems, fungal sinusitis, systemic vasculitis, or gastroesophageal reflux diseases. The diagnosis of asthma was based on history and physician's diagnosis according to the Global Initiative for Asthma 2006 guideline.<sup>11</sup> Allergic rhinitis was diagnosed on the concordance between a typical history of allergic symptoms and the atopy test. The atopic status was evaluated by skin prick test to a standard panel of aeroallergens or by using the ImmunoCAP Phadiatop test (Phadia, Uppsala, Sweden) for detecting immunoglobulin E antibodies against various common inhalant allergens. Subjective symptoms were scored on a visual analog scale (VAS) of zero to 10 as previously described.<sup>12</sup> The focus was on five major symptoms: nasal obstruction, rhinorrhea, loss of sense of smell, facial pain or pressure, and headache.<sup>12</sup> A total VAS symptom score was calculated based on the sum of these five VAS symptom domains. Endoscopy physical findings were scored according to Lanza and Kennedy.<sup>13</sup> Findings on sinus coronal computed tomography (CT) scans were graded using the Lund-Mackay CT scoring system.<sup>14</sup> Clinical data of patients are summarized in Table I.

### Sampling of Nasal Epithelial Cell

Nasal epithelial cells were scraped from the mucosa of the bilateral middle meatus using a sterile Rhino-Pro curette (Arlington Scientific Inc., Springville, UT) under an endoscope.<sup>10</sup> Scrapings were immediately transferred to a plastic tube containing 1 mL of physiological saline solution and snap-frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$  until further evaluation by reverse transcription-polymerase chain reaction (RT-PCR) assay.

### Detection of Viruses in Epithelial Cells

The RT-PCR assay of viral nucleotide was conducted as mentioned elsewhere.<sup>7,15</sup> Nucleic acid extraction from each sample was performed using an RNaprep Pure Cell/Bacteria kit (Tiangen Biotech, Beijing, China) following the manufacturer's instructions.<sup>15</sup> Briefly, about  $5 \times 10^6 - 1 \times 10^7$  cells were cracked by 600  $\mu\text{L}$  of lysis buffer RL after precipitating the cells by centrifugation. The solution was then transferred to an RNase-free filtration column CS in an RNase-free collection tube, which was centrifuged at 12,000 rpm for 2 minutes at  $4^{\circ}\text{C}$  to filter the RNA. The filtered liquid was mixed with 600  $\mu\text{L}$  of 70% ethanol and transferred to an RNase-free spin column CR3 in an RNase-free collection tube. The collection tube was then centrifuged at 12,000 rpm for 1 minute at  $4^{\circ}\text{C}$  for adsorption of the RNA. After that, buffer RW1 and DNase I were used to remove protein and DNA, respectively. Buffer RW was used for washing the RNA, and the RNA was redissolved by RNase-free ddH<sub>2</sub>O. Total RNA (1  $\mu\text{g}$ ) was reverse transcribed to cDNA using the Oligo-dT with Quantscript RT kit (Tiangen Biotech) in a final volume of 20  $\mu\text{L}$  including 1  $\times$  RT mix (RNasin, DL-dithiothre-

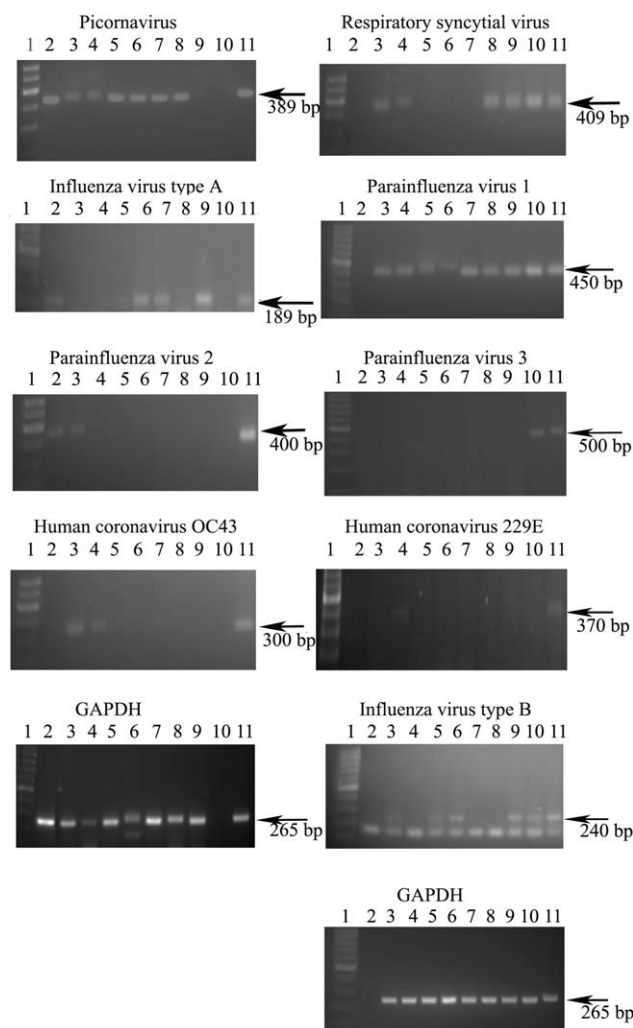


Fig. 1. Reverse transcription-polymerase chain reaction assay of respiratory viruses. The representative pictures of electrophoresis gel results are in the left panel, lane 1, molecular size marker; lanes 2 through 4, control subjects; lanes 5 through 7, chronic rhinosinusitis with nasal polyps (CRSwNP) subjects; lanes 8 and 9, chronic rhinosinusitis without nasal polyps (CRSsNP) subjects; lane 10, negative control (distilled water); lane 11, positive control for specific viruses. The representative pictures of electrophoresis gel results are in the right panel, lane 1, molecular size marker; lane 2, negative control (distilled water); lanes 3 through 5, control subjects; lanes 6 through 8, CRSwNP subjects; lanes 9 and 10, CRSsNP subjects; lane 11, positive control for specific viruses.

itol), 0.25 mM of each dNTP, 200 U quant reverse transcriptase, 1  $\mu\text{M}$  Oligo-dT, 1  $\mu\text{g}$  RNA, and additional RNase-free water.<sup>15</sup> The reverse transcription was conducted at  $37^{\circ}\text{C}$  for 60 minutes. After reverse transcription, polymerase chain reaction (PCR) was conducted as reported.<sup>7</sup> Briefly, the cDNA samples were amplified with the virus-specific primer pairs (Table II) using the 2  $\times$  PCR reagent (Tiangen Biotech). The housekeeping gene, glyceraldehyde phosphate dehydrogenase, served as an internal control for PCR. The 20  $\mu\text{L}$  PCR reaction system containing 1  $\times$  PCR reagent (1 U Taq Plus Polymerase, 250  $\mu\text{M}$  of each dNTP, 10 mM Tris-HCL, 50 mM KCL, 1.5 mM MgCl<sub>2</sub>), 0.2  $\mu\text{M}$  forward primer, 0.2  $\mu\text{M}$  reverse primer, 50 ng cDNA, and an additional 8.2  $\mu\text{L}$  RNase-free water. The PCR reaction was conducted in a thermal cycler S100 (Bio-Rad, Hercules, CA) programmed for initial cDNA synthesis and predenaturation

TABLE III.  
Identification Rate of Virus.

Virus	Control, No. (%), n = 53	CRSwNP, No. (%), n = 67	CRSsNP, No. (%), n = 61	P Value
Picornavirus	25 (49.17)	24 (35.82)	17 (27.87)	.101
Respiratory syncytial virus	10 (18.87)	10 (14.93)	5 (8.20)	.244
Influenza virus type A	14 (26.42)	10 (14.93)	10 (16.39)	.234
Influenza virus type B	6 (11.32)	8 (11.94)	12 (19.67)	.347
Parainfluenza virus 1	12 (22.64)	10 (14.93)	6 (9.84)	.167
Parainfluenza virus 2	6 (11.32)	2 (2.99)	4 (6.56)	.190
Parainfluenza virus 3	2 (3.77)	10 (14.93)	8 (13.11)	.126
Human coronavirus 229E	6 (11.32)	2 (2.99)	2 (3.28)	.089
Human coronavirus OC43	12 (22.64)	8 (11.94)	6 (9.84)	.117
Positive of any of these viruses	40 (75.47)	46 (68.66)	45 (73.77)	.678

CRSsNP = chronic rhinosinusitis without nasal polyps; CRSwNP = chronic rhinosinusitis with nasal polyps.

(94°C for 2 minutes), followed by 45 cycles of denaturation for 30 seconds at 94°C, annealing for 30 seconds at a specific temperature, and extension for 1 minute at 72°C. A final incubation was conducted for 4 minutes at 72°C. Specific virus cDNA (kindly provided by Dr. Zhan-Qiu Yang, Wuhan University, Wuhan, P. R. China) served as a positive control. Distilled water was used as a negative control. All PCR products were further assayed by agarose gel electrophoresis and ultraviolet transillumination. The identity of the PCR product was confirmed by DNA sequencing.

### Statistical Analysis

Statistical analysis was performed with SPSS 17.0 software (SPSS Inc., Chicago, IL). For continuous variable, results are expressed as medians and interquartile ranges. The Kruskal-Wallis H test was used to assess significant intergroup variability, and the Mann-Whitney *U* two-tailed test was used for between-group comparison. Differences in proportions between groups were tested by  $\chi^2$  test.  $P < .05$  was considered statistically significant.

## RESULTS

In the present study, there was no significant difference in age and sex distribution, and the proportions of concomitant asthma and allergic rhinitis among controls, CRSsNP patients, and CRSwNP patients (Table I). CRSwNP and CRSsNP patients demonstrated higher total VAS symptom scores, CT scores, and endoscopic scores compared with those seen in controls (Table I). In comparison with CRSsNP patients, CRSwNP patients displayed increased scores of CT scan and endoscopy findings (Table I).

Nine common respiratory viruses including picornavirus (most are rhinovirus), respiratory syncytial virus, influenza virus type A and B, parainfluenza virus 1, 2, and 3, and human coronavirus 229E and OC43 were detected by the PCR technique. The representative pictures of electrophoresis gel results are shown in Figure 1. As shown in Table III, virus presence could be detected in the majority of subjects studied (75.47% in controls, 68.66% in CRSwNP, and 73.77% in CRSsNP), and rhinovirus (picornavirus) was the most frequently detected species (49.17% in controls, 35.82% in CRSwNP, and 27.87%

in CRSsNP) in nasal epithelial cells of the middle meatus. There was no difference in the overall virus positive rate among the three groups studied. In addition, no difference in the detection rate of any specific individual virus could be found among these three groups, although the detection frequency of human coronavirus 229E tended to be increased in controls compared with CRSwNP and CRSsNP. We also investigated the co-occurrence of different virus species. Coinfection with more than one kind of virus was detected in 45.29% of controls, 35.82% of CRSwNP, and 32.79% of CRSsNP, and there was no significant difference among these three groups (Table IV). Finally, we subgrouped subjects into virus positive and negative subgroups and compared their clinical characteristics and clinical disease severity. We failed to find any difference in the frequency of coexistence of allergic rhinitis and asthma, and total VAS symptom scores, CT scores, and endoscopy scores between virus positive and negative subjects in the control, CRSsNP, and CRSwNP groups (Table V).

## DISCUSSION

Respiratory virus infection is considered to play an important role in the development of lower respiratory diseases, including asthma and chronic obstructive pulmonary disease. Despite the well-established link

TABLE IV.  
The Frequency of Co-colonization of Different Types of Viruses.

	Control, No. (%), n = 53	CRSwNP, No. (%), n = 67	CRSsNP, No. (%), n = 61	P Value
Single virus	16 (30.19)	22 (32.84)	25 (40.98)	0.441
Multiple viruses (>1 virus)	24 (45.29)	24 (35.82)	20 (32.79)	0.350
2 species	8 (15.09)	11 (16.42)	10 (16.39)	0.976
3 species	12 (22.64)	12 (17.91)	6 (9.84)	0.174
≥4 species	4 (7.55)	1 (1.49)	4 (6.56)	0.237

CRSsNP = chronic rhinosinusitis without nasal polyps; CRSwNP = chronic rhinosinusitis with nasal polyps.



TABLE V.  
Clinical Disease Severity in Viral Positive and Negative Subjects.

	Control			CRSwNP			CRSsNP		
	Virus Positive, n = 40	Virus Negative, n = 13	P Value	Virus Positive, n = 46	Virus Negative, n = 21	P Value	Virus Positive, n = 45	Virus Negative, n = 16	P Value
Male, no. (%)	31 (77.50)	11 (84.62)	.711	34 (73.91)	15 (71.43)	.831	33 (73.33)	11 (68.75)	.725
Age, yr, median (IQR)	30.5 (21.25–42.75)	26 (19–45.5)	.521	34.5 (22–41.25)	26 (18–39.5)	.368	28 (17.5–38.5)	29 (21–42.75)	.496
Patients with allergic rhinitis, no. (%)	6 (15)	1 (7.69)	.667	11 (23.91)	2 (9.52)	.203	11 (24.44)	4 (25)	.965
Patients with asthma, no. (%)	1 (2.5)	1 (7.69)	.434	3 (6.52)	1 (4.76)	1.000	4 (8.89)	1 (6.25)	1.000
Total VAS scores, median (IQR)	8 (5–12.75)	9 (6.5–15.5)	.604	18 (14.75–24)	18 (15.5–19.5)	.555	17 (13.5–24)	17 (13.25–21.75)	.611
CT scores, median (IQR)	0 (0–1)	0 (0–0)	.267	12 (8.75–15)	13 (8.5–17.5)	.551	12 (7–15)	8.5 (7–10.75)	.177
Endoscopy scores, median (IQR)	2 (0–2.75)	2 (0–4)	.669	6 (4–10)	8 (5–12)	.219	4 (3–5)	4 (2.25–6)	.860

CT = computed tomography; CRSsNP = chronic rhinosinusitis without nasal polyps; CRSwNP = chronic rhinosinusitis with nasal polyps; IQR = interquartile range; VAS = visual analog scale.

between CRS and chronic inflammatory lower airway diseases, the role of viruses in the pathogenesis of CRS has received little attention. Concerning the role of viruses in the pathogenesis of CRS, three hypotheses have been proposed<sup>1,8</sup>: 1) viruses are the trigger of CRS, 2) viruses are the ongoing stimulus of chronic inflammation, and 3) viruses are the causes of acute exacerbation of CRS. Similar to the two previously published studies,<sup>7,8</sup> in this study, we examined whether there is an association between respiratory virus persistence and the chronic status of CRS in subjects without signs of acute viral infection. Compared with previous studies,<sup>7,8</sup> the strengths of our current study are that a relatively larger sample size is more likely to lead to unbiased results, and epithelial cell samples from the middle meatus is more suitable to reflect the status of CRS. We found viruses present in the majority of subjects (approximately 70%) no matter whether in controls or in CRS subjects. On the contrary, Wood et al. did not detect any viruses in sinus mucosa from controls and CRS patients. This discrepancy may in part be due to the methodological difference. In Wood et al.'s study, samples were collected during the summer months when respiratory viruses are far less prevalent, and very limited subjects were examined.<sup>8</sup> In our study, we could not find any difference in the overall positive rate of virus infection, the positive rate of any specific virus infection, or the positive rate of coinfection of multiple species among controls, CRSsNP patients, and CRSwNP patients, indicating that the frequency of respiratory viral infection is not increased in CRS patients. In contrast, Cho et al. found that the detection rate of respiratory viruses was significantly increased in CRS patients compared with that seen in controls (64% vs. 30%).<sup>7</sup> The sample size in Cho et al.'s study is comparable to ours. However, in Cho et al.'s study, epithelial cells from the inferior turbinate instead of those from the middle meatus or sinus cavity were investigated for the presence of a respiratory virus. Sampling the inferior turbinate is

not appropriate, because in CRS, inflammation development takes place in the middle meatus and sinus rather than in the inferior turbinate.

In our study, we did not find any difference in clinical disease severity between subjects with and without evidence of viral infection. Based on the similar positive rate of viral infection in CRS and controls, and the similar clinical disease severity between subjects with and without viral infection, one would think that viral infection may not be the ongoing stimulus of chronic inflammation in CRS. Nevertheless, the impact of viral infection on the disease initiation and development also depends on the host response. Our previously study indicated that the innate response to viral infection may be altered in CRS patients, which may lead to subsequent exaggerated and persistent inflammation in CRS.<sup>10</sup> Therefore, to elucidate the role of viral infection in the pathogenesis of CRS, it is necessary to dissect the impact of viral infection under particular disease-related host immune response. The lack of association between viral infection and clinical disease severity does not absolutely preclude the role of viral infection in the inflammation process of CRS either, because it is possible that clinical instruments may not reflect the underlying inflammation precisely. It should also be noted that we and others did not quantify the copies of respiratory viruses. It is possible that although we did not find a difference in virus positive rate, there may be a difference in copy numbers of respiratory viruses between controls and CRS patients, which awaits further study. Another limitation of the current study is that only common respiratory viruses were studied. Further study using unbiased, highly sensitive genomic-based discovery methods, such as a pan-viral microarray platform and deep sequencing, may provide a comprehensive landscape of viral infection in CRS.<sup>16</sup> In addition, whether viruses play a role in the initiation and exacerbation of CRS also requires further study.

## CONCLUSION

Although a high frequency of viral colonization could be observed in the middle nasal meatus, there is no difference in viral infection rate among CRSsNP patients, CRSwNP patients, and controls. The contribution of the interaction of viral infection and host immunity to the pathogenesis of CRS remains to be determined.

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