

## ORIGINAL ARTICLE

## Rhinitis, Sinusitis, and Upper Airway Disease

## Endotypes of chronic rhinitis: A cluster analysis study

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## Abstract

**Background:** Chronic rhinitis (CR) is currently regarded as a syndrome, which presents as several endotypes. The aim of this study was to identify the CR endotype clusters and investigate the inflammatory patterns associated with the different endotypes.

**Methods:** A total of 259 CR patients and 20 control subjects were enrolled in this prospective study. Twelve clinical variables were analyzed using cluster analysis and five inflammatory variables were measured to investigate the inflammatory patterns associated with the different clusters.

**Results:** Six endotype clusters of CR were defined in the Chinese CR patients. Patients in cluster 1 (38.6%) were diagnosed as allergic rhinitis (AR) without asthma, and in cluster 2 (13.5%) as AR with asthma, with all demonstrating positive results for local eosinophils and high levels of local and serum IgE. Similarly, patients in cluster 3 (18.6%) were diagnosed as nonallergic rhinitis with eosinophilia syndrome (NARES) without asthma and in cluster 5 (5.0%) as NARES with asthma, with all demonstrating positive results for local eosinophils, and negative results for both local and serum IgE. Patients in cluster 4 (4.6%) were diagnosed as local allergic rhinitis and showed positive results for local eosinophils and local IgE, but negative results for serum IgE, whereas patients in cluster 6 (19.7%) were diagnosed as idiopathic rhinitis because of high symptoms scores, but negative findings for local eosinophils, local IgE, and serum IgE.

**Conclusions:** Chinese CR patients may be clustered into six endotypes with different inflammatory patterns, which may help in delivering individualized treatment.

## KEYWORDS

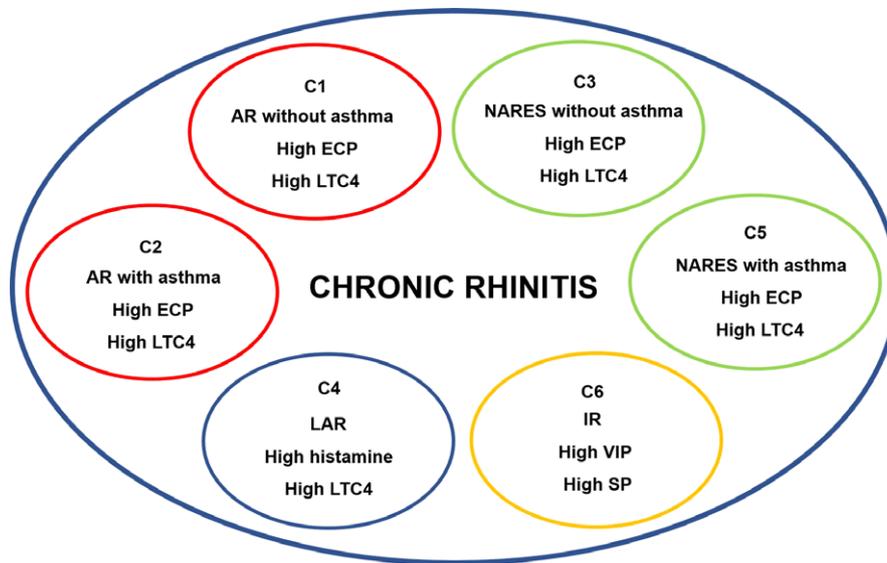
chronic rhinitis, cluster analysis, endotype, local IgE, serum IgE

## 1 | INTRODUCTION

Chronic rhinitis (CR) is defined as a symptomatic inflammation of the nasal mucosa, characterized by two or more symptoms, including

nasal congestion, anterior or posterior rhinorrhea, sneezing and itching for at least 1 hour daily and for more than 12 weeks per year.<sup>1,2</sup> CR is one of the most common diseases with a considerable financial burden globally.<sup>3,4</sup> The morbidity of CR is high, with earlier studies estimating up to about 30% of the total population being affected.<sup>1,5</sup> Moreover, CR has been shown to be associated with chronic rhinosinusitis and is a risk factor for asthma. Indeed, CR adversely impacts

**Abbreviations:** AR, allergic rhinitis; ECP, eosinophil cationic protein; IR, idiopathic rhinitis; LAR, local allergic rhinitis; LTC<sub>4</sub>, leukotriene C<sub>4</sub>; NARES, nonallergic rhinitis with eosinophilia syndrome; SP, substance P; VIP, vasoactive intestinal peptide.



## GRAPHICAL ABSTRACT

Chinese CR patients could be clustered into six endotype clusters, including: (1) Allergic rhinitis without asthma; (2) Allergic rhinitis with asthma; (3) nonallergic rhinitis with eosinophilia syndrome (NARES) without asthma; (4) local allergic rhinitis; (5) NARES with asthma; (6) idiopathic rhinitis. Clusters 1, 2, 3 and 5 demonstrated significantly higher levels of eosinophil cationic protein and leukotriene C4 (LTC4); whereas cluster 4 demonstrated significantly higher levels of histamine and LTC4. Similarly, Cluster 6 demonstrated significantly higher levels of vasoactive intestinal peptide and substance P.

the quality of life and leads to decreased productivity, sleep impairment, and psychological impairment.<sup>3,6</sup>

Previously, while CR was generally subcategorized into two phenotypes, allergic rhinitis (AR) and nonallergic rhinitis, CR is currently regarded as a syndrome, which presents under an “umbrella” as several phenotypes with different pathogenic pathways.<sup>3,7</sup> These endotypes/phenotypes are overlapping, dynamic, and rendering clear-cut definitions difficult.<sup>3</sup> Chronic rhinitis is a heterogeneous disease, with distinct pathophysiologic processes. For example, AR is a Th2 inflammation of nasal mucosa mediated by IgE antibody and nonallergic rhinitis may be associated with either a neurogenic pathway, autonomic imbalance, age, pregnancy, occupation, or drugs.<sup>1,8</sup> Therefore, the different subtypes of CR need distinct therapeutic strategies involving different therapeutic responses and prognoses.<sup>7</sup> Thus, the phenotyping of CR associated with the different pathophysiologic mechanisms and distinct medical treatments is urgently needed.

From a clinical perspective, the clinical indicators are more suitable to phenotyping chronic rhinitis because these are easier to determine/observe in daily clinical practice. In this respect, Papadopoulos and Hellings have subcategorized chronic rhinitis with two similar methods.<sup>1,3</sup> Both of these phenotype categories are commonly used around the world and have greatly promoted the diagnosis and treatment of subjects with these CR phenotypes. In contrast, phenotyping CR using distinct biomarker/s is more difficult presently due to the scarcity of appropriate biomarkers.<sup>9</sup>

Although several classifications of rhinitis endotypes/phenotypes have been proposed to date,<sup>1,3,9</sup> there is some degree of subjectivity, little scientific basis in the description of some of these endotypes/phenotypes, and some of them lack clinical relevance.<sup>7</sup> However, it is

possible that these limitations may be overcome by use of cluster analysis. The aim of this study was therefore to perform cluster analysis of CR patients to identify the different endotypes and investigate the inflammatory patterns of these endotypes.

## 2 | MATERIALS AND METHODS

### 2.1 | Study design and subjects

This prospective single center study was conducted from February 1, 2017, to August 31, 2017. Subjects suspected to have rhinitis based on the presence of common symptoms of nasal obstruction, rhinorrhea, sneezing, and itching were recruited consecutively from the allergy-rhinology outpatient clinic of Beijing TongRen Hospital. Each subject completed a questionnaire at recruitment to record their demographic data, nasal symptom severity, and asthma history, and was then assessed for sensitization to relevant aeroallergens by measurement of specific IgE (sIgE) in both serum and nasal secretions. Diagnosis of AR was based on criteria of the Allergic Rhinitis and its Impact on Asthma (ARIA) consensus statement.<sup>5</sup> Diagnosis of asthma was based on the history and lung function examination. Healthy subjects without any nasal disease and patients with nasal septum deviation, cerebrospinal fluid leak (CSF-leak), or pituitary tumor with normal nasal mucosa in the ethmoid sinus, but without chronic rhinitis, were recruited as controls.

The exclusion criteria for the study included chronic rhinosinusitis and/or nasal polyposis as defined by the European position paper on rhinosinusitis and nasal polyposis,<sup>10</sup> any respiratory infection in the previous 4 weeks, and CT scan showed opacification in the nasal cavity or sinuses. Patients who had taken systemic corticosteroids

over the past 3 months, intranasal corticosteroids over the past 4 weeks, antihistamines over the past 2 weeks, and vasoconstrictors over the past 1 week were also excluded.

The study was conducted in full accordance with Declaration of Helsinki and approved by the Medical Ethics Committee of Beijing TongRen Hospital. All patients also provided written informed consent prior to entry into the study and collection of data. In the case of children, of whom 11 were enrolled, their parents or guardians provided written informed consent prior to entry into the study and collection of any data. Firstly, the aim of the study was explained to both the children and their parents or guardians, and voluntary participation in the study was emphasized. Subsequently, full details were provided of the duration of the study, the clinical and laboratory procedures to be undertaken, and the need to collect blood and nasal secretion samples for analysis.

## 2.2 | Visual analogue scale

The severity of nasal symptoms, including nasal obstruction, anterior or posterior rhinorrhea (watery, mucous or purulent), sneezing and nasal/eye itching, was recorded using a visual analogue scale (VAS) of 10 cm. Each symptom was categorized as "mild" (VAS: 0-3 cm), "moderate" (VAS: >3-7 cm), or "severe" (VAS >7 cm).

## 2.3 | Fractional exhaled nitric oxide

Fractional exhaled nitric oxide (FeNO) was measured using a nitric oxide analyzer (Niox; Aerocrine, Solna, Sweden) at a flow rate of 50 mL/s through the oral cavity. The gas was continuously routed into the analyzer via a side port. The procedure was repeated three times and a mean concentration of FeNO was calculated.

## 2.4 | Serum sIgE

Serum sIgE levels to common aeroallergens were determined using the fluoroenzyme immunoassay (UniCAP, Uppsala, Sweden), with a value for serum sIgE  $\geq 0.35$  kU/L regarded as positive. The sIgE examination was performed with a panel of allergens including *Dermatophagoides farinae* (*Der f*), *Dermatophagoides pteronyssinus* (*Der p*), *Candida albicans*, mugwort, *Penicillium notatum*, *Cladosporium*, *Alternaria*, and *Aspergillus*. These allergens were selected on the basis that mite and mugwort are two most prevalent aeroallergens in China.<sup>11</sup> Furthermore, based on evidence from daily clinical practice during the period when this study was conducted, we noted that the fungal allergy rate was also high and thus the panel of fungal allergens was investigated additionally.

## 2.5 | Nasal secretions: collection and Local sIgE measurement

Nasal secretions were collected using sinus packs and processed as described previously.<sup>12</sup> All samples were collected from patients when they presented to our allergy outpatient clinic with symptoms, during

or out of the season. We obtained all the samples during symptomatic period. All sponges were stored at 4°C for at least 2 hours and then transferred to a 5 mL BD syringe. The bulk of the nasal secretion was forced out of the sponges using the piston of the syringe and centrifuged at 1500 g for 15 minutes at 4°C. The supernatants were separated and stored in aliquots at -20°C until analysis for the presence of sIgE and other inflammatory mediators. At the time of assay, an aliquot was also assessed to make sure there was no contamination or disturbance of the other factors. Local sIgE in nasal secretions was assessed using the fluoroenzyme immunoassay (UniCAP, Uppsala, Sweden) and the panel of aeroallergens as for serum. Again, a value for sIgE  $\geq 0.35$  kU/L was regarded as positive.

## 2.6 | Nasal allergen provocation test

Nasal allergen provocation test (NAPT) was performed to make a diagnosis for LAR, according to the EAACI position paper on the standardization of nasal allergen challenges.<sup>13</sup> The diluent was delivered to the inferior turbinate using a nebulizer with a fixed volume of 100  $\mu$ L per puff. Solutions were applied to the subjects with breath holding after a deep inspiration to avoid allergen being inhaled into lower airways. After a 30-minute adaptation period, the subjects were given 100  $\mu$ L of diluent followed by rhinomanometry to exclude nasal hyper-reactivity. Then, *Der f* allergen extract and mugwort extract (0.004, 0.04, 0.4, and 4  $\mu$ g/mL, WOLWOPHARMA, Zhejiang, China)<sup>14,15</sup> were applied in both nasal cavities at less than 10 minutes intervals, until a positive reaction was observed at less than 10 minutes intervals. The clinical symptoms and the change in nasal airway resistance (NAR) were reassessed at a transnasal pressure difference of 150 Pa with active rhinomanometry 30 minutes after NAPT. Total symptom score was assessed according to the earlier study.<sup>16</sup> NAPT was considered positive when the total symptom score was  $\geq 4$  or nasal airflow was reduced by 60% or more from the baseline level. NAPT was also considered positive when the total symptom score was  $\geq 3$  and nasal airflow was reduced by 20% or more from baseline.

## 2.7 | Histological evaluation of nasal mucosa

Samples of nasal mucosa were obtained from 84 CR patients and from control subjects with nasal septum deviation (n = 21), cerebrospinal fluid (n = 4), or pituitary tumor (n = 2) during surgery, and processed for histological evaluation using H&E staining. All samples were processed by an independent pathologist, who was blinded to the clinical diagnosis and characteristics of the patients and assessed the numbers and different types of inflammatory cell (eosinophils, neutrophils, plasma cells, and lymphocytes) by bright-field light microscopy (BX51, Olympus, Japan) at  $\times 400$  magnification. The eosinophils in the nasal secretion were counted according to a recent study by Howarth et al.<sup>17</sup>

## 2.8 | Measurement of inflammatory mediators

The nasal secretions obtained from the participants at baseline were assessed for the levels of eosinophil cationic protein (ECP) and

**TABLE 1** The demographic characteristics and clinical variables of chronic rhinitis patients

	Control	Cluster 1 Allergic rhinitis without asthma	Cluster 2 Allergic rhinitis with asthma	Cluster 3 NARES without asthma	Cluster 4 Local allergic rhinitis	Cluster 5 NARES with asthma	Cluster 6 Idiopathic rhinitis	P value
No. of subjects	20	100	35	48	12	13	51	
Age (y)	38.2 ± 8.7	33.6 ± 7.1	31.9 ± 14.5	37.4 ± 11.2	38.3 ± 13.7	40 ± 9.9	35.6 ± 11.6	ns
Gender (M/F)	8/12	58/42	21/14	28/20	7/5	8/5	31/20	ns
Asthma	0	0	35	0	0	13	2	<0.001
FeNO	7.8 (2-13.9)	10.9 (1-25)	43.8 (10-112)	12.1 (9-17)	3.4 (1-11)	39.2 (10-56)	7.8 (2-18)	<0.001
Nasal obstruction	1 (0-2)	4 (3-7)	4 (3-8)	5 (3-8)	3(1-5)	5 (3-7)	6 (3-10)	<0.05
Rhinorrhea	0	3 (2-6)	4 (3-6)	3 (1-6)	3 (1-5)	4 (2-6)	4 (2-7)	ns
Nasal itching	0	3 (0-6)	2 (0-5)	2 (0-3)	2 (0-5)	2 (0-3)	5 (3-8)	<0.05
Sneezing	0	3 (1-7)	3 (1-5)	3 (1-5)	2 (1-5)	3 (2-5)	4 (2-7)	ns
VAS	1	15	16	17	13	18	21	<0.05
Local eosinophilia	0	2	2	3	2	3	0	ns
Serum IgE	0.02 (0-0.35)	73.1 (0.35-111.3)	68.2 (0.35-103.7)	0.02 (0-0.35)	0.01 (0-0.35)	23.9 (0-118.9)	0.01 (0-0.35)	<0.001
Local IgE	0.01 (0-0.35)	42.8 (0.35-100.1)	65.6 (0.35-81.2)	0.03 (0-0.35)	87.5 (17.5-201.5)	0.15 (0-17.5)	0.01 (0-0.35)	<0.001

F, female; FeNO, fractional exhaled nitric oxide; M, male; NARES, nonallergic rhinitis with eosinophilia syndrome; VAS, visual analogue scale.

Data are presented as mean ± SD.

Statistical significance was evaluated by one-way analysis of variance, chi-square test, and Tukey's test.

histamine using commercial ELISA kits from CUSABIO (Wuhan, Hubei province, China), and the levels of leukotriene C4 (LTC4), substance P (SP), and vasoactive intestinal peptide (VIP) using commercial ELISA kits from R&D Systems (Minneapolis, MN). Similarly, the concentration of total protein in each sample was measured using commercial ELISA kits from Beyotime (Beijing, China), and the concentration of each inflammatory mediator was expressed as ng/g protein. When the level of a mediator measured was below the detection level in the initial measurement, the results of that particular sample were excluded.

## 2.9 | Statistical analysis

Hierarchical clustering method with Euclidean similarity measure and Ward minimum-variance linkage was performed using R3.1.2 (The R Foundation for Statistical Computing, <http://www.r-project.org/>), and the result was rendered as a heat map drawn with R. Normality standardization was performed by computing Z score for each variable, such that all variables were of similar dimension in further analysis.

The clinical parameters between the clusters were compared by one-way analysis of variance, chi-square test, and Tukey's test. The secreted mediators were analyzed by a Kruskal-Wallis H test to assess significant intergroup variability among more than two groups and a Mann-Whitney U two-tailed test was used for between-group comparison. All the statistics between groups were performed by using SPSS version 22.0 (IBM Corp., Armonk, NY) and GraphPad Prism 7.0 software (GraphPad Software, Inc., La Jolla, CA). Bonferroni's multiple comparison test was applied to correct for

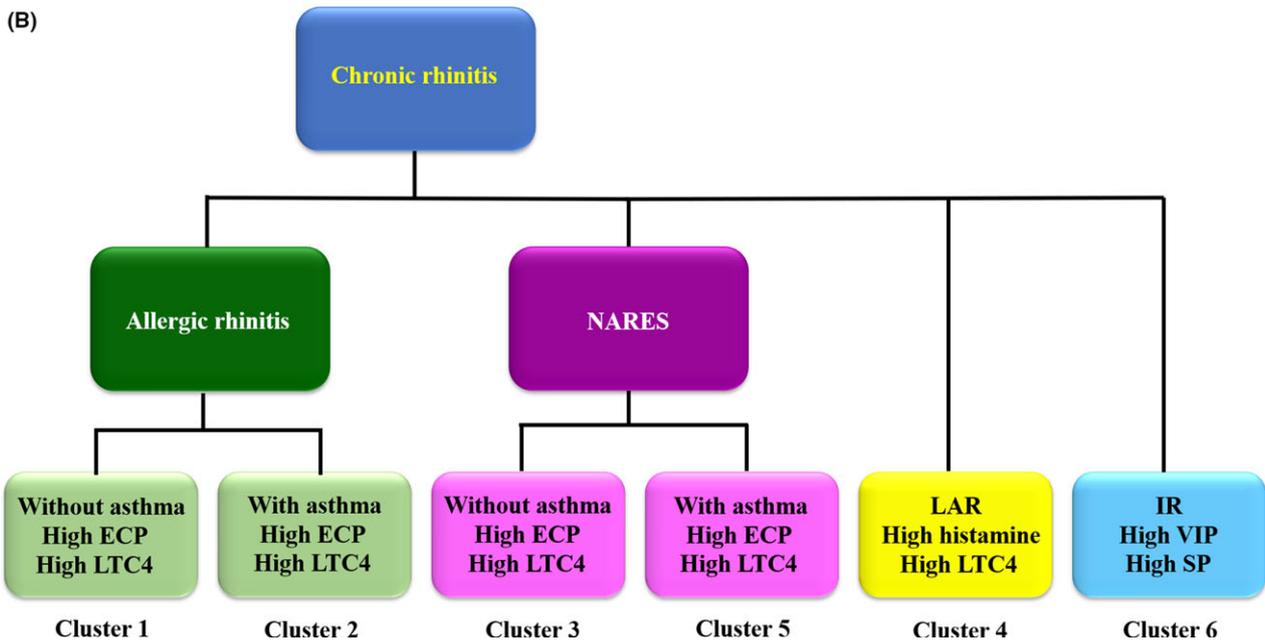
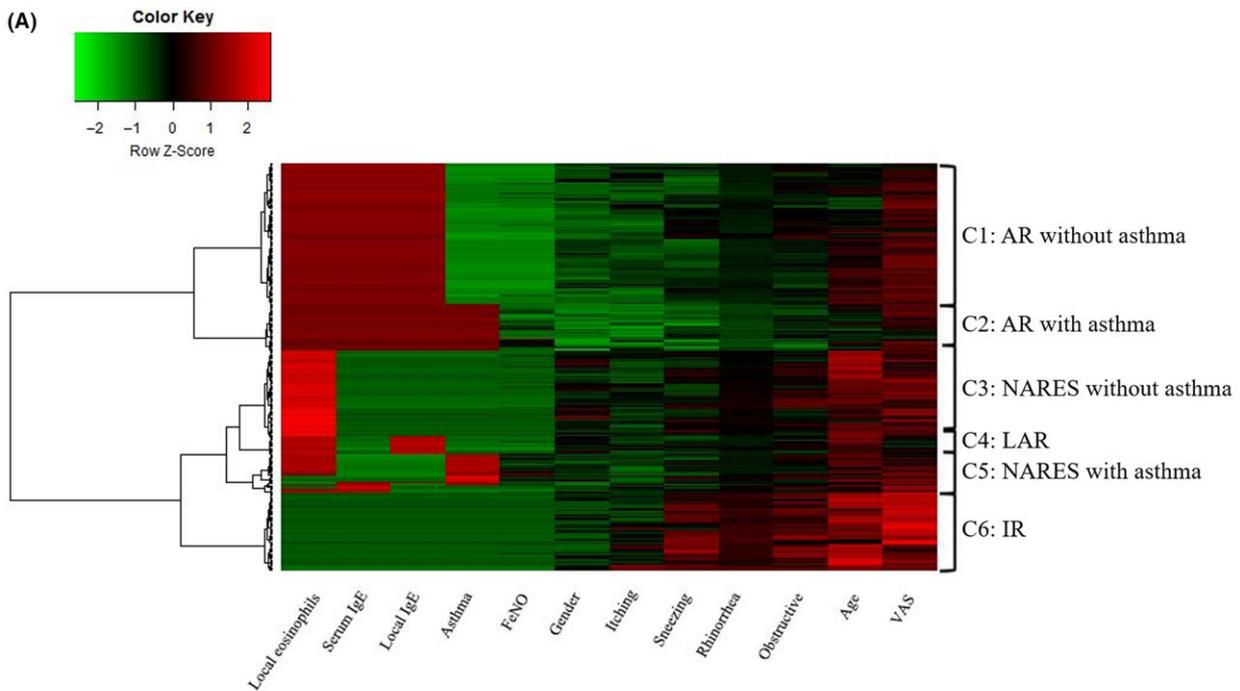
significance. Data were expressed as median and interquartile range, unless otherwise specified. Statistical significance was set at a P value ≤0.05.

## 3 | RESULTS

Overall, 259 (153 males and 106 females) patients and 20 controls were recruited into the study. The age of the participants ranged from 8 years to 58 years (mean = 36.1 years). Twelve variables were used for the cluster analysis, including age, gender, asthma history, FeNO, serum IgE, local nasal IgE, local eosinophils, nasal obstruction score, rhinorrhea score, nasal itching score, sneezing score, and VAS (Table 1). Based on this analysis, six subject clusters were identified (Figure 1).

### 3.1 | Characteristics of clusters

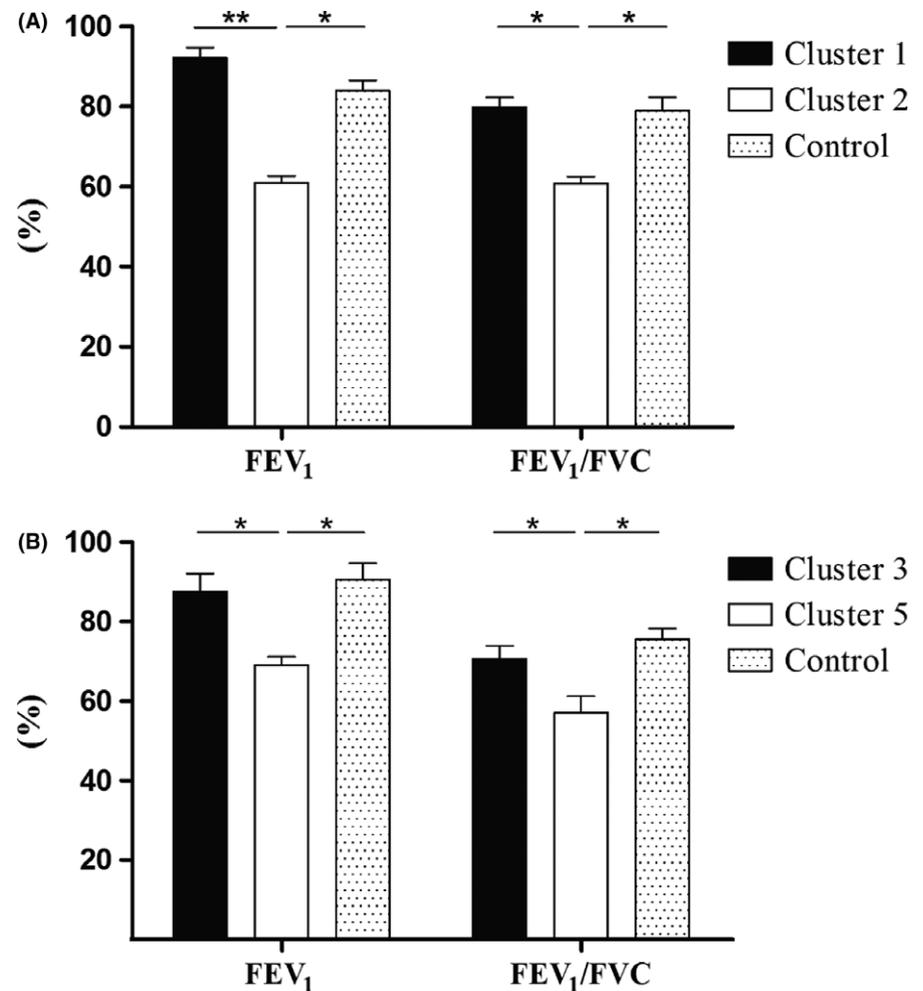
Clusters 1 (allergic rhinitis [AR] without asthma) and 2 (AR with asthma) comprised a total of 135 patients; of which 58 males and 42 females (mean age = 33.6 years) were present in cluster 1 and 21 males and 14 females (mean age = 31.9 years) were present in cluster 2. There was no significant difference between these two clusters with respect to the VAS, and both were positive for local eosinophils and higher levels of serum IgE and local IgE. However, all 35 patients in cluster 2 had concomitant asthma (Table 1) and demonstrated significantly lower FEV<sub>1</sub> predictive and FEV<sub>1</sub>/FVC ratio (Figure 2A) and significantly higher FeNO levels (Table 1), compared to cluster 1 and controls subjects.



**FIGURE 1** A, Hierarchical clustering of twelve clinical variables. Shown is a heat map of clustering clinical characteristics in chronic rhinitis. Each row represents one patient, and the range is shown in each column, with green being low and red being high. Left, dendrogram showing similarity of groups. Right, six clusters (C1-C6) are indicated by vertical bars. AR, allergic rhinitis; FeNO, fractional exhaled nitric oxide; IR, idiopathic rhinitis; LAR, local allergic rhinitis; NARES, nonallergic rhinitis with eosinophilia syndrome; VAS, visual analogue scale. B, The main characteristics of different clusters. ECP, eosinophil cationic protein; LTC4, leukotriene C4; SP, substance P; VIP, vasoactive intestinal peptide

Similarly, clusters 3 (nonallergic rhinitis with eosinophilia syndrome [NARES] without asthma) and 5 (NARES with asthma) included a total of 61 patients, with 28 males and 20 females (mean age = 37.4 years) in cluster 3, and eight males and five females (mean age = 40.0 years) in Cluster 5. There was no significant difference between these two clusters with regard to the

VAS, and both clusters were positive for local eosinophils and negative for serum IgE and local IgE. Also, all 13 patients in cluster 5 had concomitant asthma (Table 1) and demonstrated significantly lower FEV<sub>1</sub> predictive, FEV<sub>1</sub>/FVC ratio (Figure 2B) and significantly higher FeNO levels compared to cluster 3 and control subjects (Table 1).



**FIGURE 2** The comparison of FEV<sub>1</sub> predictive, FEV<sub>1</sub>/FVC ratio between cluster 1 and cluster 2 (A) and cluster 3 and cluster 5 (B)

Overall, 12 patients (seven males and five females, mean age = 38.3 years) fell into cluster 4 (local allergic rhinitis [LAR]). None of these patients had a history of asthma and had the lowest VAS and the higher level of local eosinophils. Moreover, the patients in cluster 4 demonstrated positive results for local IgE and negative results for serum IgE (Table 1). Based on the features of this cluster, we performed nasal allergen provocation test in the 12 patients using *Dermatophagoides farinae* (*Der f*) and mugwort, and found that all the patients had positive results at 4 µg/mL for total symptoms score and nasal endoscopic examination, following nasal provocation with *Der f* (Figure 3), but not following nasal provocation with diluent or mugwort.

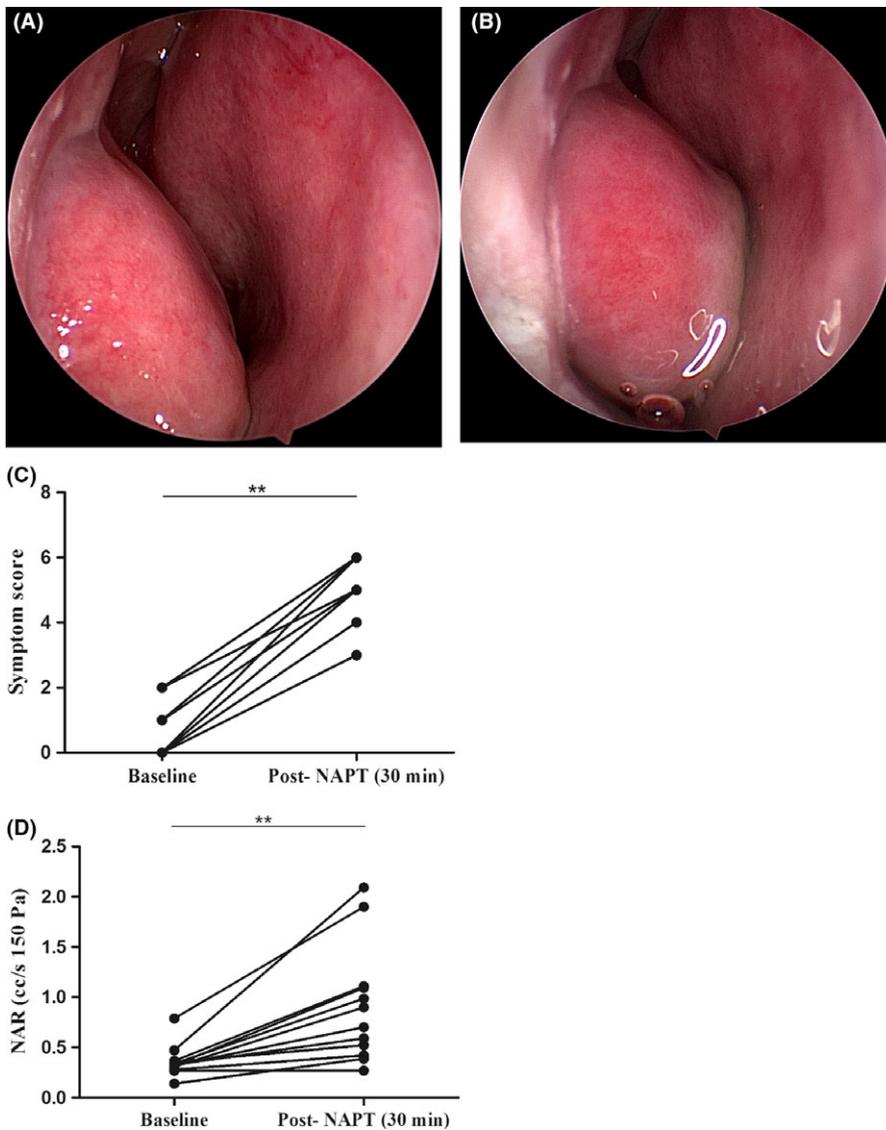
Cluster 6 (idiopathic rhinitis [IR]) included a total of 51 patients (31 males and 20 females, mean age = 35.6 years). None of these patients had a history of asthma and all demonstrated negative results for local eosinophilic, local IgE, and serum IgE. However, these patients had the highest VAS (Table 1).

Figure 1B provided an overview of the major findings for the mediators assessed in the six clusters. Patients in clusters 1, 2, 3, and 5 had significantly higher levels of ECP and LTC<sub>4</sub>, whereas patients in cluster 4 had significantly higher level of histamine. Similarly, patients in cluster 6 had significantly higher levels of VIP and SP (Figure 4).

Representative histological features of the nasal mucosa in control subjects and patients from the different clusters were shown in Figure 5. Eosinophils were present in tissue of patients with allergic rhinitis (Figure 5B) and NARES (Figure 5C), but not in tissues from control subjects (Figure 5A) or from patients with IR (Figure 5D).

## 4 | DISCUSSION

Chronic rhinitis is a prevalent upper airway disease. It can occur concurrently with other airway diseases and leads to a considerable financial and social burden.<sup>1,5,18</sup> However, the precise diagnosis and treatment of the disease in the clinic are not fully optimized. The diagnostic criteria for specifically allergic rhinitis are well-documented globally and easily implemented by most doctors to make the correct diagnosis. In contrast, nonallergic rhinitis presents a bigger problem because it is often underestimated in daily activity, despite more than 200 million individuals being affected by nonallergic rhinitis worldwide.<sup>19</sup> Moreover, despite the similar clinical presentations between different phenotypes, it is not easy to specifically diagnose nonallergic rhinitis due to the variability in pathophysiologic mechanisms, despite the similar clinical



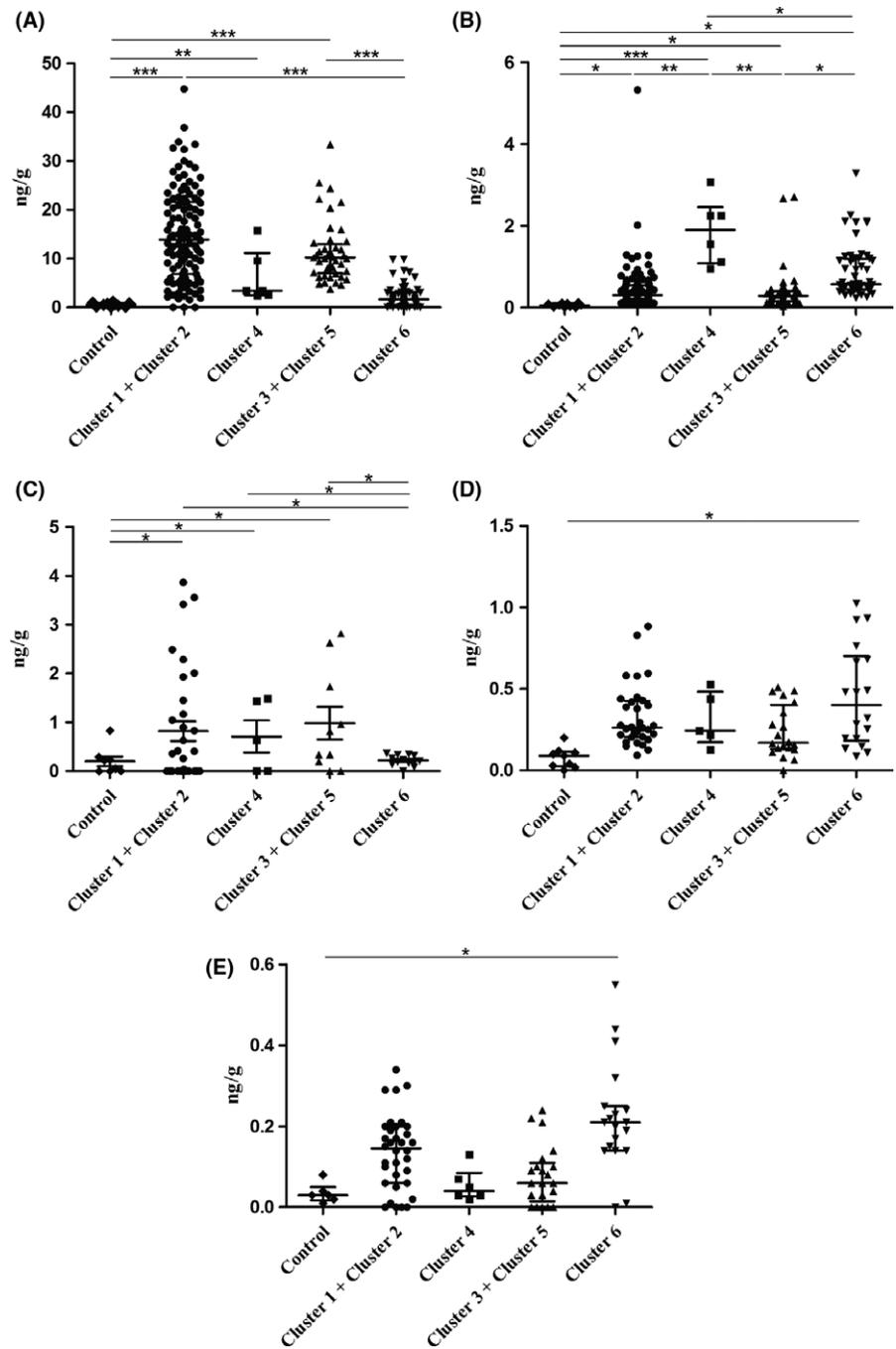
**FIGURE 3** Representative effect of nasal allergen provocation test (NAPT) in patients from cluster 4. The inferior turbinate and nasal mucosa were normal before NAPT (A). Thirty minutes after NAPT, there was swelling of the inferior turbinate and production of large quantities of watery secretions in the nasal cavity (B). NAPT also significantly increased the symptom score (C) and nasal airway resistance (NAR) (D) 30 min after NAPT

presentations between different phenotypes.<sup>20,21</sup> In this regard while an early study by Papadopoulos et al<sup>3</sup> have subcategorized chronic rhinitis, which partially overlapped another study by Helings et al<sup>1</sup> subcategorized chronic rhinitis based on their clinical presentations. This type of phenotyping, however, is oversimplified and somewhat non-specific because phenotypes were based on medical history, physical examination, and *in vivo/in vitro* investigations of a few inflammatory proteins being measured to verify the endotypes. In the present study, we used unsupervised hierarchical cluster analysis to determine the endotypes more specifically based on 12 different clinical variables. This analysis demonstrated six endotype clusters with different clinical indicators, which were verified using different methods.

Clusters 1 and 2 included 100 and 35 patients, respectively, who according to the ARIA guideline criteria, were diagnosed as being AR with confirmed local production of IgE in the nasal mucosa.<sup>20,21</sup> Furthermore, according to the ARIA guidelines and medical history, the patients in cluster 2 were diagnosed as AR

with asthma. This diagnosis was in accordance with epidemiological evidence, which has consistently shown the co-existence of rhinitis and asthma.<sup>22</sup> Our findings for the significantly greater production of ECP, histamine, LTC<sub>4</sub>, and VIP in patients from clusters 1 and 2 compared with the control subjects are also in accordance with the well-documented “one airway, one disease” concept showing a correlation between the upper and lower airway allergy symptoms.<sup>3</sup> Indeed, these findings were also in line with the Th2-mediated inflammatory mechanisms in AR and asthma, involving respiratory mucosal inflammation with eosinophils, and synthesis of ECP and LTC<sub>4</sub>.<sup>23</sup>

Nonallergic rhinitis with eosinophilia syndrome was first described by Jacobs and colleagues in 1981.<sup>24</sup> Although there was no consensus about the diagnostic criteria for NARES, the high level of eosinophilic cells in nasal smears was a common feature and varies from 5% to 25%.<sup>25-28</sup> In the present study, clusters 3 and 5 included 48 and 13 patients, respectively, with VAS scores of 17 and 18, respectively. Furthermore, although both the serum

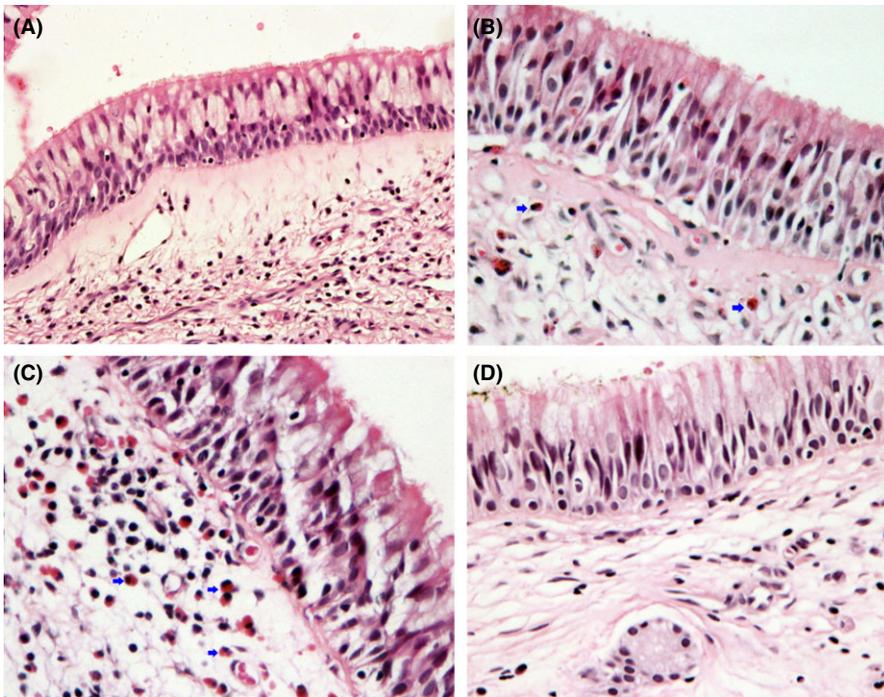


**FIGURE 4** Measurements of inflammatory mediators and neuropeptides between different clusters. A, eosinophil cationic protein (ECP); B, histamine; C, leukotriene C4 (LTC4); D, vasoactive intestinal peptide (VIP); E, substance P (SP)

IgE and local IgE were negative in these clusters (0.02 and 23.9 kU/L, 0.03 and 0.15 kU/L, respectively), the levels of eosinophils were the highest in these clusters. Thus, we diagnosed these 61 patients as NARES patients. The morbidity of NARES in this cohort was higher than that described in a former study,<sup>26</sup> and it was possible that this discordance in our findings may be due to the fewer patients in this study. However, the most prominent difference between cluster 3 and cluster 5 was that all 13 patients classified into cluster 5 had the asthma symptoms. These findings suggested that non-IgE-mediated eosinophilic inflammation may manifest in both the upper and lower airways as NARES and eosinophilic asthma, respectively. Although the morbidity of asthma in

the present cohort of 61 NARES patients was lower than in an earlier study,<sup>29</sup> the levels of ECP in these clusters were found to be significantly higher than the control group. This is in accordance with the former study.<sup>30</sup> Indeed, clusters 1, 2, 3, and 5, which were all found to have significantly higher levels of ECP and LTC4 than the control group and cluster 6. Thus, this finding suggested that the use of anti-leukotrienes, which could effectively reduce eosinophilic inflammation, also needs to be emphasized for the CR patients besides the use of intranasal corticosteroids (INS) in NARES patients.

Despite characterization of only 12 patients in cluster 4, this was a clinically interesting cluster because all the demographic and



**FIGURE 5** The nasal mucosa of control subjects (A; n = 27), and patients with allergic rhinitis (B; n = 49), nonallergic rhinitis with eosinophilia syndrome (NARES) (C; n = 24) and idiopathic rhinitis (IR) (D; n = 11). The blue arrows indicate the eosinophils in the mucosa. (H&E,  $\times 400$  magnification)

clinical characteristics, apart from the local IgE levels, employed for clustering were negative. An earlier study suggested that mite and mugwort were two most prevalent aeroallergens in China.<sup>11</sup> Based on this finding, we performed NAPT with *Der f* and mugwort, in these 12 patients and demonstrated that all had a positive NAPT for *Der f*. This finding was in accordance with the concept of “entropy” which was first proposed by Powe and colleagues.<sup>20,21</sup> It was possible, however, that the results for these patients in cluster 4 may be due to these patients being recruited from February to May, while mugwort was the main aeroallergen in Autumn in Beijing (duration from later July to end of September). Thus, based on the findings of Rondon et al,<sup>31</sup> these patients might be classified as LAR patients, particularly because of (a) local production of IgE, (b) Th2 inflammatory pattern in nasal secretion when exposed to allergens, and (c) positive results of NAPT without systemic atopy. It was interesting that this earlier study reported that LAR affects 25.7% of the rhinitis population,<sup>31</sup> whereas the present study demonstrated that only 5% (12 patients) met the criteria for LAR. It is possible that this difference may be a result of differences in ethnicity, because most studies investigating LAR have been performed in white patients in Europe. Furthermore, none of the patients in cluster 4 in the present study had concomitant asthma, whereas LAR was frequently associated with asthma in the study of Rondon and colleagues.<sup>31</sup> Unfortunately, there were no other studies of LAR in Asian patients for comparison with the present study. However, the VAS was lowest in this cluster because the disease was mostly limited to the middle meatus and the other parts of nasal mucosa were normal, while levels of histamine were the highest. From this point, it was likely that besides INS, antihistamines might also be useful to relieve the nasal irritation in this group of patients. The finding that ECP and

LTC4 levels in these patients were significantly higher than in the control subjects is in accordance with the former study<sup>31</sup> and suggested that anti-leukotriene therapy should be added to INS therapy as required in individual CR patients.

Cluster 6, including a total of 51 patients, appears to be somewhat different because despite the VAS being the highest of all the clusters, the total IgE, local IgE, and local eosinophilia were all negative in this cluster. Thus, based on the diagnosis of exclusion, we diagnosed these patients as idiopathic rhinitis (IR).<sup>3</sup> IR was the most prevalent NAR subtype and is in accordance with the findings from several studies which have reported that IR represented about 70% of NAR cases.<sup>3,32</sup> The pathogenesis of IR has been suggested to be nonallergic, but rather due to a disorder of peptidergic neural system.<sup>33</sup> In the present study, both SP and VIP levels in this cluster were significantly higher than in the control subjects. Indeed, a recent study has suggested that nociceptive TRPV1-substance P signaling pathway was upregulated in IR patients.<sup>34</sup> Similarly, SP has been shown to induce the release of histamine, the main protein associated with nasal irritation,<sup>35</sup> and VIP has been shown to be associated with nasal hyperresponsiveness.<sup>36</sup> Thus, from the treatment aspect, although INS might not be the best treatment of choice for IR patients, several studies have suggested that capsaicin might theoretically be an alternative.<sup>37,38</sup>

In summary, this study for the first time provides a useful way of objectively defining at least six endotype clusters of chronic rhinitis, including a cluster 1 comprising allergic rhinitis without asthma, a cluster 2 comprising allergic rhinitis with asthma, a cluster 3 comprising NARES without asthma, a cluster 4 comprising local allergic rhinitis, a cluster 5 comprising NARES with asthma, and cluster 6

comprising idiopathic rhinitis. Furthermore, based on the different inflammatory patterns defining these clusters, the study provides the opportunity for providing the most appropriate and effective individualized treatment strategies for CR patients.

## CONFLICT OF INTEREST

All authors declare that they have no conflict of interests.

## AUTHOR CONTRIBUTIONS

Y.M. prepared the manuscript and performed the statistical analysis. H.L., K.W., X.W., and F.C. collected the clinical data and samples. X.C. analyzed the samples. C.W. participated in collection of the clinical data and samples and was involved in the preparation of the manuscript. L.Z. designed the study and participated in the interpretation and discussion of the data.

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