Research article

Increased prevalence of *CFTR* variants and susceptibility to CRS: A real-world study based on Chinese children

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ABSTRACT

Background: Chronic Rhinosinusitis is a common disease in children. The main function of CFTR is to maintain the thickness of the mucous layer on the surface of the nasal mucosa. *CFTR* disease-causing variant can cause CFTR protein dysfunction and induce or aggravate chronic infection. However, the carrying status of the *CFTR* variants in the Chinese population is not clear.
Objective: To study the frequency and variants of *CFTR* in Chinese children with CRS and to analyze the *CFTR* variants and the clinical characteristics and susceptibility to CRS.
Methods: Whole Exome Sequencing was performed to analyze the *CFTR* genes in a total of 106 CRS children from the Chinese mainland area. The *CFTR* variants, frequency and clinical data were summarized and analyzed.
Results: A total of 31 *CFTR* variants were detected, of which the carrying rate of 7 sites was significantly higher than that of the population database. 88 patients carried more than 2 variants. 37 people carried variants (MAF < 0.05), of which 91.89% had a history of recurrent upper respiratory infections, 16 had nasal polyps, 5 had bronchiectasis, and 1 was diagnosed with CF-related disorders.
Conclusion: The carrying rate of *CFTR* variants in Chinese CRS children increased, and the highest rates of variants (MAF < 0.05) are p.I556V, p. E217G, c.1210-12[T]. Carrying multiple *CFTR* variants, especially p.E217G, p.I807 M, p.V920L and c.1210-12[T] may lead to increased susceptibility to CRS. There are CF-related disorders in patients with CRS.

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1. Introduction

Chronic rhinosinusitis (CRS) is a common disease characterized by chronic infection of the nasal epithelium. The prevalence rate of clinical-based CRS can reach 3.0%–6.4% [1]. CRS is more common in children after an upper respiratory tract infection, with a prevalence rate of 4% [2]. It is a syndrome that affects the quality of life of children. However, some children with CRS still cannot achieve the desired outcome after regular treatment. Among the diseases associated with CRS, Cystic Fibrosis (CF) is a multisystem disorder caused by cystic fibrosis transmembrane conductance regulator (*CFTR*) disease-causing variant [3]. So far, more than 2000 different *CFTR* variants have been reported (http://www.genet.sickkids.on.ca). They are divided into six categories according to their effect on proteins. Class I: abolish the production of the full-length CFTR polypeptide; Class II: impairs protein trafficking/maturation; Class III: impairs regulation of channel gating; Class IV: anion permeation through the open channel pore; Class V: reduced quantity of CFTR protein; Class VI: affects the lifetime of the channel protein in the apical membrane [4]. The most common pathogenic variant is F508del (Class II, III and VI), which is also the most common disease-causing variant in Caucasians [5]. However, many studies have shown that the frequency of *CFTR* variants is different in different populations and regions [6]. According to the previous studies of patients with CF in the Chinese population, it was found that the five most common disease-causing variant were p.G970D, c. 1766+5G>T, p.I1023R, p.L88X and p.Q98R [7,8]. In China, CF is considered a rare disease. At present, there are only about 90 Chinese CF patients reported in the literature [7–9], but most of these patients have been reported in the last 5 years, suggesting that the carrying rate of *CFTR* variants and even the prevalence of CF may not be as rare as imagined [7]. Moreover, some studies have shown that carriers of *CFTR* variants have a higher probability of developing CRS than the general population [10,11]. In addition, the decrease in the function of CFTR protein caused by *CFTR* variants, which leads to CRS, usually begins in childhood [12]. Raman's study shows that the incidence of *CFTR* variants increases in children with CRS who are not diagnosed with CF [13]. However, the carrying rate and common variants of the *CFTR* in the Chinese population are not clear, especially for Chinese children with CRS. This is the first study to detect *CFTR* in the Chinese CRS population and provides an in-depth analysis of the *CFTR* carrying situation of Chinese CRS children, in order to understand the relationship between *CFTR* variant gene carrying and disease. Understanding this information will be of great help for the treatment and prognosis of children with refractory CRS.

2. Methods

2.1. Patient enrollment

This study retrospectively analyzed the patients who were admitted to the Hospital from August 2020 to January 2022. According to the diagnostic criteria of CRS for children in EPOS 2020 [14], 121 patients met the following symptoms and signs: they must have two or more symptoms, one of which is nasal congestion or nasal secretion (anterior/posterior nasal drip); the other is either facial pain and a swelling sensation, and/or cough. These symptoms should last for more than 12 weeks. All of them received regular drug treatment in the outpatient clinic for 12 weeks (including nasal saline irrigation, nasal corticosteroids, anti-leukotrienes, and some of them were given muco-active drugs), and their symptoms did not improve significantly or show recurrence. 15 of them were excluded because of malignant tumors, autoimmune diseases, or severe congenital malformations. 106 children with CRS were enrolled in this
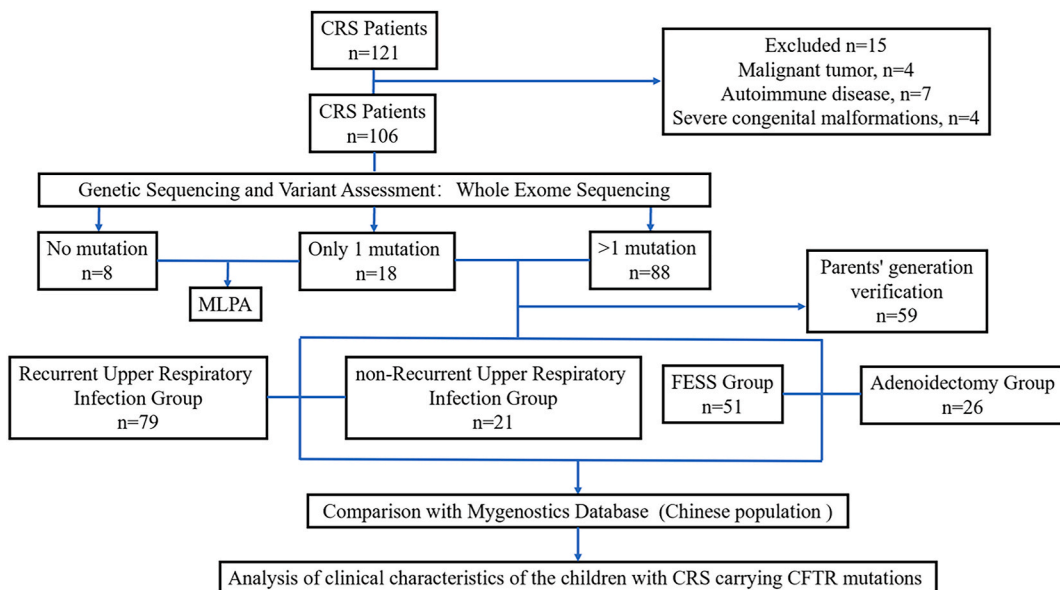

Fig. 1. A flow chart of the research.

study (Fig. 1). The medical history, blood examination, imaging examination and family history of these 106 patients were collected and analyzed. According to the history of respiratory infection (3–5 years old: upper respiratory tract infection 6 times per year, lower respiratory tract infection 2 times per year. 6–12 years old: upper respiratory tract infection 5 times per year, lower respiratory tract infection 2 times per year. The interval between the two respiratory tract infections was at least 7 days), patients were divided into two groups: recurrent upper respiratory infection group (n = 79) and non-upper respiratory infection group (n = 27). Of the 106 patients, 77 underwent surgery. According to the mode of operation, the 77 patients were divided into Functional Endoscopic Sinus Surgery (FESS) group (n = 51) and Adenoidectomy group (n = 26).

The study was approved by the Ethics Committee of the Hospital and the informed consent of all subjects was obtained (2020-Z-175).

2.2. Genetic sequencing, variant assessment and copy number variations

Whole Exome Sequencing (WES) was performed to analyze the *CFTR* according to the manufacturer's instructions.

2.2.1. Library preparation

In brief, genomic DNA was extracted, hybridized, and enriched. Firstly, 1 μg of genomic DNA was extracted from 200 μL of peripheral blood, using a Qiagen DNA Blood Midi/Mini kit (Qiagen GmbH, Hilden, Germany) following the manufacturer's protocol. 50 ng of DNA was interrupted to 200bp around by fragmentation enzymes. The DNA fragments were then end repaired, and the 3′end was added one A base. Secondly, the DNA fragments were ligated with barcoded sequencing adaptors, and fragments without PCR amplification. The DNA fragments were hybridized and captured by Berry's Nano WES Human Exome V1.0 (Berry genomics, Beijing, China) according to the manufacturer's standard operating procedure. The hybrid products were eluted and collected, and then subjected to PCR amplification and the purification.

Finally, Novaseq6000 platform (Illumina, San Diego, USA), with 150bp pair-end sequencing mode, was used for sequencing the genomic DNA of the family. Raw image files were processed using CASAVA v1.82 for base calling and generating raw data.

2.2.2. Data analysis

The sequencing reads were aligned to the human reference genome (hg38/GRCh38) using the Burrows–Wheeler Aligner tool and PCR duplicates were removed by using Picard v1.57 (http://picard.sourceforge.net/). The average depth of *CFTR* gene sequencing was 82.77. The coverage of the CDS region of *CFTR* is 100%, and the coverage of exon region (including 5′UTR and 3′UTR) is 82.7% (Tables S1–3). The Verita Trekker® Variants Detection System by Berry Genomics and the third-party software GATK (https://software.broadinstitute.org/gatk/) were employed for variant calling. Variant annotation and interpretation were conducted by ANNOVAR and the Enliven® Variants Annotation Interpretation System authorized by Berry Genomics.

For the Copy Number Variations (CNV) part, a comprehensive tool called Sprinkle was developed by Berry Genomics was used for CNV calling. It included XHMM PCA method to remove sequencing noise, CNVKit fix module to perform GC and bias correction, and then copy number calculation and CNV identification were performed in exons and long segment areas.

2.3. Parental generation verification and MLPA

200μl was taken from the whole blood samples of the subjects' parents for the first-generation verification of *CFTR*. A blood/tissue/cell genome extraction kit (Tiangen biochemical Technology Co., Ltd., DP304-03) was used to extract genomic DNA. 27 coding exons of *CFTR* and FLANKING sequences were sequenced by 3730XL sequencer (ABI; California, USA). The sequencing traces were analyzed by Codon Code Aligner software (Codon Code Aligner Corporation; Centerville, MA, USA) and compared with the reference sequence NM_00492.4. General rearrangement tests are performed using commercial MLPA kits (SALSA®P091-D1 CFTR, MRC-Holland; Amsterdam, The Netherlands). The results were analyzed by Coffalyser software (MRC-Holland; Amsterdam, The Netherlands).

2.4. Statistical analysis

All data were calculated and analyzed by SPSS26.0 statistical software. The significance of the difference between groups was analyzed by $\chi 2$ test or Fisher's exact test. The continuous variables were tested by Shapiro-Wilk test for normal distribution, and the results are expressed in the form of median ±standard deviation (median ±SD). An independent sample *t*-test (double tail) or nonparametric Mann-Whitney *U* test was used to compare the results between the two groups. The results were considered to be significantly different if the P value was less than 0.05.

CRS is a common disease with unclear genetic pattern and complex etiology in children. According to the definition of Minor Allele Frequency (MAF), the MAF of the common variation in the population is more than 0.05 [15,16]. In this study, we calculated the variant allelic frequency (V. A. F) f of all the detected variants and compared them with MAF. We pay more attention to the variants with MAF < 0.05 in the *CFTR* detection in Chinese CRS children. In order to facilitate the identification and expression in this paper, these variants are collectively referred to as "variants (MAF < 0.05)".

3. Results

3.1. Clinical characteristics of patients

This study included 106 Han Chinese children in the Chinese mainland area, including 78 males (73.58%) and 28 females (26.24%), ranging in age from 1 year 9 months–15 years 7 months, with a median age of 8 years 7 months. The medical history and clinical information of 106 children with CRS were analyzed retrospectively (Table 1). 74.53% (n = 79) of the patients had a history of recurrent upper respiratory infections, and 34 patients' guardians participated in the survey of whether the patients were exposed to secondhand smoke, of which 22 children (64.7%) were exposed to it for more than 8 h a day. 26.4% of the children had relatives with allergic diseases, more than 50% had relatives with respiratory diseases, 2 patients' mothers had reproductive diseases, and all guardians denied that there were CF patients in the family, or that the patients had a history of pancreas-related diseases and reproductive system diseases. According to the EPOS 2020, 36 patients were diagnosed with nasal polyps: 13 of them with Antro-choanal Polyp (ACP), 23 of them with Chronic Sinusitis with Nasal Polyp (CRSwNP); 35 patients completed the sweat chloride levels test (SCLs), and 8 of them had SCLs >60 mmol/L. Finally, 26 patients underwent an adenoidectomy, 51 patients underwent FESS and 29 patients chose to take drugs in the outpatient clinic instead of operation. 43 patients had a lung function test before the operation, and 41 of them had a bronchial provocation test, of which 25 had decreased pulmonary function (58.14%) and 11 had positive bronchial provocation test (26.83%). All guardians denied that the patients had a history of asthma or other chronic respiratory diseases. The WES and CNV results of 106 patients with CRS showed that 83.02% carried *CFTR* variants, the number of sites varied

Table 1
The clinical characteristics of patients with CRS.

Characteristic	CRS
	n = 106
Sex (M/F)	78/28
Age (y)	8.36 ± 2.60
Medical History	
Duration of disease	24.92 ± 17.35
Allergy, n (%)	39 (n = 67, 58.21%)
Recurrent Upper Respiratory Infection, n (%)	79 (74.53%)
History of exposure to secondhand smoke, n (%)	22 (n = 34, 64.70%)[a]
Family history	
Allergic Diseases	28 (26.4%)
Respiratory Diseases	44 (51.51%)
Digestive Diseases	17 (16.34%)
Endocrine Diseases	2 (1.89%)
Reproductive Diseases	2 (1.89%)[b]
Blood Test	
Eos in blood (%)	2.59 ± 1.80
Lymphocytes in blood (%)	41.13 ± 10.67
Neu in blood (%)	48.35 ± 12.49
Serum IgE (IU/ml)	201.02 ± 39.14 (n = 58)
Serum IL-6 (pg/ml)	2.98 ± 2.26 (n = 45)
Imaging	
Lund-Mackay score	11.35 ± 6.94 (n = 66)
Lund-Kennedy score	6.94 ± 1.81 (n = 89)
Diagnosis	
ACP, n (%)	13 (12.26%)
CRSwNP, n (%)	23 (21.70%)
CRSsNP, n (%)	70 (66.04%)
Operation	
Adenoidectomy, n (%)	26 (24.53%)
FESS, n (%)	51 (48.11%)
Without Operation, n (%)	29 (27.36%)
Detection of Exhaled Nitric Oxide	
FeNO50	13.39 ± 9.72 (n = 40)
FnNO10	35.49 ± 491.82 (n = 45)
Lung Function	
Normal, n (%)	18 (n = 43, 41.86%)
Decrease, n (%)	25 (n = 43, 58.14%)
Bronchial Challenge Test, Positive, n (%)	11 (n = 41, 26.83%)
Sweat Chloride Levels	50.80 ± 17.18 (n = 35)
Carrying *CFTR* variants, n (%)	88 (83.02%)
Parents' Verification, n (%)	59 (55.66%)

Data are expressed as numbers (%) and means ± standard deviation.
[a] The patient was exposed to secondhand smoke for more than 8 h a day.
[b] Two mothers provided a history of reproductive system diseases, one with polycystic ovary and the other with tubal obstruction.

from 1 to 14, 8 patients carried only one *CFTR* variants, and only 18 patients had no *CFTR* variants. Although the CNV test has been able to confirm that there are no large rearrangements, insertions, and deletions in all subjects, in order to avoid false negative results, we still tested the MLPA of the *CFTR* in these 26 people, and the results once again confirmed that there was no large fragment deletion of *CFTR*. The parents of 59 patients underwent first-generation verification of *CFTR*, including 8 patients with SCLs >60 mmol/L.

3.2. Subgroup analysis of clinical features

We divided the patients into two subgroups. First, according to the history of respiratory infection, we divided the patients into the recurrent upper respiratory infection group and non-upper respiratory infection group (Table 2). Among the patients in the recurrent upper respiratory infection group, 45.57% were associated with nasal polyps, 64.56% underwent FESS and 13.92% underwent an adenoidectomy, which was significantly higher than those in the non-upper respiratory tract infection group.

Then, the surgical patients with CRS were divided into the FESS group and adenoidectomy group (Table 3). The age of the patients who underwent FESS were older than those who underwent an adenoidectomy. At the same time, the Lund-Kennedy scores were higher. In the FESS group, 70.59% of the children had nasal polyps, and all the patients had a history of recurrent upper respiratory infection. In contrast, only 11 people (42.30%) in the adenoidectomy group had a history of respiratory infection, which was significantly lower than the FESS group.

3.3. The number of CFTR variants carried by CRS patients

A total of 31 *CFTR* variants were detected in this study, of which 1 was located in the promoter, 14 in the exon and 16 in the intron. We chose the American College of Medical Genetics and Genomics (ACMG) [17] to evaluate the disease-causing potential of the variants, and 17 of them were rated as VUS (Variant of Uncertain Significance), 1 was rated as Likely Pathogenic and the rest as Benign. Among all the detected variants, the three variants (MAF < 0.05) with the highest variant allelic frequency (V. A. F) were p.I556V, p. E217G and c.1210-12[T]. In order to get a better understanding of the relationship between the variants and the disease, we used the ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), Human Gene Mutation Database (HGMD: http://www.hgmd.cf.ac.uk/ac/index.php), CFTR2 (https://www.cftr2.org/) and CFTR-France https://cftr.iurc.montp.inserm.fr/cftr databases to summarize the variant information. We found 27 variants in ClinVar; 14 variants in the HGMD database, and 11 of them were rated as DM and DM? sites. Only 6 variants were recorded in the CFTR2. 19 variants were recorded in the CFTR-France database, of which c.1666A>G (p.Ile556Val;

Table 2

Analysis of clinical characteristics of CRS children with or without recurrent upper respiratory infection.

Characteristic	Recurrent Upper Respiratory Infection	non-Recurrent Upper Respiratory Infection	P-value
	Group n = 79	Group n = 27	
Sex (M/F)	59/20	19/8	0.661
Age (y)	8.38 ± 0.31	8.31 ± 0.41	0.918
Medical History			
Duration of disease	25.09 ± 2.07	24.44 ± 2.75	0.683
Allergy, n (%)	34 (n = 59, 57.63%)	5 (n = 8, 62.5%)	0.036
Blood Test			
Eos in blood (%)	2.57 ± 0.21	2.67 ± 0.29	0.629
Lymphocytes in blood (%)	41,14 ± 1.27	41.11 ± 1.71	0.993
Neu in blood (%)	48.16 ± 1.52	48.92 ± 1.76	0.928
Serum IgE (IU/ml)	199.24 ± 40.90 (n = 56)	251 ± 68 (n = 2)	0.186
Serum IL-6 (pg/ml)	3.00 ± 0.34 (n = 44)	1.80 (n = 1)	0.875
Imaging			
Lund-Mackay score	11.59 ± 0.88 (n = 63)	6.33 ± 0.30 (n = 3)	0.223
Lund-Kennedy score	7.17 ± 0.22 (n = 72)	6.00 ± 0.24 (n = 17)	0.016
Diagnosis			
ACP, n (%)	13 (16.46%)	0	< 0.001*
CRSwNP, n (%)	23 (29.11%)	0	
CRSsNP, n (%)	43 (54.43%)	27 (100%)	
Operation			
Adenoidectomy, n (%)	11 (13.92%)	15 (55.56%)	< 0.001*
FESS, n (%)	51 (64.56%)	/(n = 0)	
Without Operation, n (%)	17 (21.52%)	12 (44.44%)	
Sweat Chloride Levels	50.80 ± 20.90 (n = 35)	/(n = 0)	/
Detection of Exhaled Nitric Oxide			
FeNO50	15.02 ± 1.68 (n = 39)	8.0 (n = 1)	0.374
FnNO10	365.38 ± 72.89 (n = 44)	274 (n = 1)	0.758
Lung Function			
Normal, n (%)	18 (n = 43, 41.86%)	/(n = 0)	/
Decrease, n (%)	25 (n = 43, 58.14%)	/(n = 0)	/
Bronchial Challenge Test, Positive, n (%)	11 (n = 41, 26.83%)	/(n = 0)	/

Data are expressed as numbers (%) and means ± standard deviation.

Table 3
The clinical characteristics of CRS patients with different operation.

Characteristic	FESS Group	Adenoidectomy Group	P-value
	n = 51	n = 26	
Sex (M/F)	40/11	17/9	0.217
Age (y)	9.54 ± 0.33	7.35 ± 0.48	< 0.001*
Medical History			
Duration of disease	23.22 ± 2.86	25.27 ± 2.87	0.06
Allergy, n (%)	27 (n = 43, 62.79%)	5 (n = 6, 83.33%)	0.005
Recurrent Upper Respiratory Infection, n (%)	51 (100%)	11 (42.30%)	< 0.001*
Blood Test			
Eos in blood (%)	2.56 ± 0.28	2.60 ± 0.29	0.575
Lymphocytes in blood (%)	39.81 ± 1.33	40.72 ± 2.38	0.718
Neu in blood (%)	48.91 ± 1.86	49.59 ± 2.41	0.763
Serum IgE (IU/ml)	149.32 ± 32.98 (n = 45)	154.24 ± 39.46 (n = 5)	0.190
Serum IL-6 (pg/ml)	3.01 ± 0.35 (n = 37)	4.73 ± 2.75 (n = 3)	0.583
Imaging			
Lund-Mackay score	11.63 ± 0.94 (n = 51)	12.00 ± 10.00 (n = 2)	0.833
Lund-Kennedy score	7.69 ± 0.27 (n = 51)	7.00 ± 1.00 (n = 26)	< 0.001*
Diagnosis			
ACP, n (%)	13 (25.49%)	0	< 0.001*
CRSwNP, n (%)	23 (45.10%)	0	
CRSsNP, n (%)	15 (29.41%)	26 (100%)	
Sweat Chloride Levels	51.94 ± 2.96 (n = 33)	32.00 ± 1.00 (n = 2)	0.033
Detection of Exhaled Nitric Oxide			
FeNO50	14.52 ± 1.72 (n = 34)	/(n = 0)	/
FnNO10	402.51 ± 85.80 (n = 35)	63.50 ± 53.50 (n = 2)	0.122
Lung Function			
Normal, n (%)	15 (n = 36, 41.67%)	1 (n = 2, 50.00%)	0.084
Decrease, n (%)	21 (n = 36, 58.33%)	1 (n = 2, 50.00%)	
Bronchial Challenge Test, Positive, n (%)	9 (n = 35, 25.71%)	1 (n = 2, 50.00%)	

Data are expressed as numbers (%) and means ± standard deviation.

legacy:I556V) and c.2421A>G (p.Ile807Met; legacy:I807 M) have been reported to cause CFTR-related disorders [18]; c. 1210-12[T] is 5T, which is considered "Varying clinical consequence" in CFTR2 and CFTR-France database (Table 4).

The V. A. F of *CFTR* variants in CRS patients were calculated and compared with the carrying rates in the gnomAD_genome_EAS database (n = 9435). Only 25 variants could be found. However, the geographical distribution and ethnic proportion of the personnel included in the database in East Asia are not clear. For example, out of 9435 people, it is not known how many of them are from the Han nationality in Chinese mainland area. Considering the limitations of the database, we cited the Chinese population database provided by Beijing Mygenostics co., LTD (n = 46,648), but there were still 2 variants without data in the Mygenostics Database. c.1766 + 82dup is not included in any of the above databases. The Mygenostics Database showed that there were 7 single nucleotide polymorphisms (SNPs) with >0.05. Statistical analysis of the carrying rate from the database and the V. A. F of this study showed that there were 7 variants in the CRS patients which is significantly higher than in the Chinese population (P < 0.05): 3 in the exons and 4 in the introns (Table 4).

3.4. CFTR variants in CRS patients with different clinical features

After excluding the SNPs (MAF > 0.05), isosemantic and some Benign variants, 19 CFTR variants were analyzed. First of all, the V. A. F of *CFTR* variants in CRS patients with and without recurrent upper respiratory infection were compared. 18 sites were detected in the upper respiratory infection group, while only 3 were detected in the non-upper respiratory infection group, and no statistical difference was found between the two groups. However, by comparing the carrying rate of the Mygenostics Database with the two groups respectively, it was found that 7 variants in the recurrent upper respiratory infection group were significantly higher than those in the database (P < 0.05). On the other hand, there was no significant difference between the non-recurrent upper respiratory infection group and the Mygenostics Database. After that, the V. A. F of *CFTR* variants in the FESS group and the adenoidectomy group were compared. 15 sites were detected in the FESS group, but only 4 were detected in the adenoidectomy group, and no statistical difference was found between the two groups. Comparing the carrying rate of the Mygenostics Database with the two groups respectively, it was found that 4 sites in the FESS group were significantly higher than those in the database (P < 0.05) (Table 5).

3.5. Prediction of harmfulness of CFTR variants carried by CRS children

3.5.1. The location of CFTR variants

According to the location information of the *CFTR* variants, we marked the 31 sites on the gene schematic diagram (Fig. 2). At the same time, we marked some of the detected exons in their domain (Fig. 3): p.I125T and p.E217G in TMD1; p.I556V in NBD1;p.F650L and p.I807 M in RD;p.V920L in TMD2.

Table 4
Statistical analysis of the V. A. F of *CFTR* variants and the carrying rate from the mygenostics database and classification in different databases.

NO.	HGVS.c	ACMG	ACMG Criteria	V.A.F	Mygenostics Database	P-value	OR	95% CI	gnomAD_genome_EAS	Clinvar	HGMD	CFTR2	CFTR-France
1	c.-152G>C	VUS	PM2	0.004717	NA	/	/	/	/	Uncertain significance	DM?	/	/
2	c.-8G>C	Benign	BA1	0.070755	0.059885	0.648379	1.167579	0.671221:2.030984	0.063218	Benign/Likely benign	DM?	non CF-causing	non disease-causing
3	c.91C>T (p. Arg31Cys; legacy:R31C)	VUS	PM5_Strong	0.004717	0.004588	0.590651	1.680072	0.336052:8.399410	0.004817	Benign;Likely benign; Uncertain significance	DM	non CF-causing	unclassified
4	c.374T>C (p. Ile125Thr; legacy: I125T)	VUS	PM1	0.014151	0.010644	1	1.206590	0.345969:4.208050	0.009811	Likely benign; Uncertain significance	DM	/	/
5	**c.650A>G (p. Glu217Gly; legacy: E217G)#**	VUS	PM1	0.028302	0.012691	**0.038790**	2.681548	1.224311:5.873257	0.006705	Benign;Likely benign; Uncertain significance#	DM	/	unclassified
6	c.1408G>A (p. Val470Met; legacy: V470 M)	Benign	PM1 BA1	0.495283	0.422172	0.058774	1.313673	0.991784:1.740031	0.436660	Benign/Likely benign	DFP	non CF-causing	non disease-causing
7	c.1666A>G (p.Ile556Val; legacy:I556V)	VUS	PM1	0.037736	0.045372	0.728053	0.841512	0.405323:1.747104	0.049635	Benign;Likely benign; Pathogenic	DM	/	CFTR-RD-causing
8	c.1950C>A (p. Phe650Leu; legacy:F650L)	VUS	PM1 PM2	0.004717	0.001125	0.197731	6.847533	1.358061:34.526204	/	Uncertain significance	DM?	/	/
9	**c.2421A>G (p. Ile807Met; legacy: I807M) #**	VUS	PM1	0.004717	0.000086	**0.018522**	85.078431	14.910224:485.461458	0	Benign; Likely benign; Pathogenic; Uncertain significance	DM	non CF-causing	CFTR-RD-causing
10	c.2562T>G (p.Thr854 =)	Benign	BA1 BP7	0.490566	0.426106	0.109761	1.266421	0.956040:1.677566	0.435701	Benign/Likely benign	1.R 2.FP	non CF-causing	non disease-causing
11	**c.2758G>T (p. Val920Leu; legacy: V920L) #**	Likely pathogenic	PM1 PM2 PM5 PP3	0.004717	0.000011	**0.004146**	482.147287	49.931180:4655.728175	0	Pathogenic; Uncertain significance	DM	/	unclassified
12	c.3870A>G (p.Pro1290 =)	Benign	BA1 BP7	0.004717	0.004663	0.596587	1.652943	0.330640:8.263409	0.004619	Benign	/	/	non disease-causing
13	c.4389G>A (p.Gln1463 =)	Benign	BA1 BP7	0.023585	0.016260	0.251885	1.755377	0.750796:4.104108	0.018533	Benign/Likely benign	DFP	/	non disease-causing
14	c.*1043A>C	VUS	NA	0.004717	0.003218	0.466387	2.393380	0.476439:12.023086	0.003655	Benign/Likely benign	DM?	/	/
15	c.*1251C>T	Benign	BA1	0.004717	0.016928	0.389267	0.449731	0.089985:2.247674	0.018897	Benign	/	/	unclassified

Table 4 (*continued*)

NO.	HGVS.c	ACMG	ACMG Criteria	V.A.F	Mygenostics Database	P-value	OR	95% CI	gnomAD_genome_EAS	Clinvar	HGMD	CFTR2	CFTR-France
16	**c.164 + 16T>C#**	VUS	PM2	0.004717	0.000032	**0.008275**	206.630122	30.355351:1406.539723	/	/	/	/	/
17	c.489 + 91A>G	VUS	BS1	0.004717	0.001994	0.322152	3.870171	0.771331:19.418644	0.001914	Likely benign	/	/	/
18	c.1210-12[T]	VUS	PP3	0.023585	0.000526	0.073112	2.515358	1.075065:5.885245	0.024946	Pathogenic; Likely pathogenic; Uncertain significance; Likely benign	DM	varying clinical consequence	varying clinical consequence
19	c.1393-61A>G	Benign	BA1	0.471698	0.411261	0.144254	1.239755	0.935353:1.643222	0.433974	Benign	/	/	non disease-causing
20	**c.1680-113C>T#**	VUS	NA	0.018868	0.003929	**0.007743**	5.977812	2.331054:15.329642	0.006736	/	/	/	/
21	c.1680-870T>A	Benign	BA1	0.240566	0.334093	0.000232	0.538867	0.382278:0.759597	0.422171	Benign	/	/	non disease-causing
22	c.1766 + 152T>A	Benign	BA1	0.490566	0.352002	0.000158	1.730968	1.306522:2.293301	0.437500	Benign	/	/	non disease-causing
23	c.1766 + 82dup	VUS	PM2	0.004717	NA	/	/	/	/	/	/	/	/
24	c.2619 + 86_2619 + 87del	Benign	BA1	0.221698	0.227266	0.264480	0.809542	0.566797:1.156247	0.416634	Benign	/	/	/
25	c.2620-180G>A	VUS	NA	0.004717	0.002955	0.438225	2.607438	0.519241:13.093577	0.003471	Likely benign	/	/	/
26	c.3139 + 42A>T	Benign	BA1	0.004717	0.000043	0.453015	2.490461	0.497482:12.467551	0.003657	Benign	/	/	non disease-causing
27	c.3140-92T>C	Benign	BA1	0.004717	0.004212	0.559639	1.830196	0.365989:9.152212	0.004621	Benign	/	/	non disease-causing
28	c.3717 + 45G>A	VUS	BS1	0.004717	0.007289	1	1.055044	0.211250:5.269183	0.017561	Benign/Likely benign	/	/	unclassified
29	**c.3717 + 92A>G#**	VUS	PM2	0.004717	0.000011	**0.004146**	482.147287	49.931180:4655.728175	/	/	/	/	/
30	c.3718-2476G>A	VUS	PM2	0.004717	0.000712	0.131511	10.762671	2.103231:55.074818	0.000577	Likely benign	/	/	/
31	**c.3874-200G>A#**	Benign	BA1	0.018868	0.001329	**0.000196**	17.451088	6.406267:47.537891	/	Benign	/	/	non disease-causing

Reference sequence: NM_00492.4; The gnomAD version: v3.1.1; VUS: Variant of Uncertain Significance; DM: Disease-causing Mutation; DM?: likely Disease-causing Mutation; FP: in vitro or in vivo Functional Polymorphism; DFP: Disease-associated polymorphism with additional Functional Evidence. CFTR-RD: CFTR-related disorders.

V.A.F: Variant Allelic Frequency of *CFTR* variants in this study.

P-value: Statistical analysis of the *CFTR* carrying rate from the Mygenostics database and the V.A.F of CRS children.

#: The V. A. F of *CFTR* variants in CRS children was significantly higher than that in the Mygenostics database, P < 0.05.

3.5.2. Prediction of harmfulness of variants in exons and introns

First of all, we selected the SIFT tool (http://sift-dna.org) and Polymorphism Phenotypingv2 software to predict the harmfulness of carrying these *CFTR* variants, and the results of some exon sites were shown in Table S4. The smaller the SIFT-Score or the higher the Polyphen2-Score, the more likely it is to cause disease at the specific site. Therefore, p.R31C and p.I807 M are high risk sites for disease. Although the variant genes in the intron region do not transcribe amino acids, they also play an important role in regulation. Therefore, we chose Splice AI software to predict whether intron sites may cause splicing changes. The value of Delta Score (0,1) represents the probability that the variant affects splicing. A Delta Score of 0.2 indicates that the variant may affect splicing and has pathogenicity, >0.5 indicates pathogenicity, and >0.8 indicates high pathogenicity (**Table S5**). In the results, only the Delta Score of c. 3874-200G>A is 0.23, which means the possibility of the variant affecting splicing is still very low. Moreover, the predicted result of c.1766 + 82dup is not affecting splicing.

3.6. Who carries the sites that may cause the disease?

Among the 106 children with CRS, 51 of them were found to carry SNPs (MAF > 0.05). 5 patients had SCLs >60 mmol/L, but the results of their parents' first-generation verification suggested that the mutations only came from the father or mother. 37 patients carried non-SNPs, and all of them were heterozygous. The parents of 21 of them agreed to take blood samples for first-generation verification (56.75% of the overall first-generation verification). We summarized the clinical characteristics of 37 children with CRS (Table S6, Fig. 4). Among the 37 patients, 34 (91.89%) had a recurrent upper respiratory infection, and 33 (89.18%) had a history of CRS longer than 12 months. 14 patients provided a family history of respiratory, digestive, and allergic diseases. 29 patients (78.37%) underwent surgery, 22 patients underwent FESS, 4 of whom had a history of acute sinusitis with orbital cellulitis (cured after operation and anti-infective treatment), 16 patients suffered from nasal polyps, 4 of whom had revision surgery within 1 year of the first operation. 18 patients had a pulmonary function test before operation, of which 9 decreased, 17 had a bronchodilation test, and 4 were positive (Table 6). There were 19 patients who had a pulmonary CT examination and 5 of them were diagnosed with bronchiectasis (4 were diagnosed with CRSwNP and 1 was diagnosed with ACP). All the 5 patients were treated and followed up by a respiratory physician and otorhinolaryngologists at the same time after the operation, but 3 of them still had revision surgery 1 year after the operation. We also measured the levels of blood glucose, blood amylase and lipase in these 37 patients, as well as the level of fecal elastase, and no abnormal results were found. 15 of them had a sweat chloride level test, and only 3 of them were >60 mmol/L. They were all CRSwNP patients, and 2 of them had bronchiectasis, but their parents' verification results did not support the diagnosis of CF. Patient 41 (CRSwNP) was diagnosed with CF-related disorders because he carried two VUS sites which came from his father and mother, and his nasal polyps showed active proliferation, but his lung function did not decline and the result of SCLs was lower than 60 mmol/L (58 mmol/L).

4. Discussion

CRS and CF are related diseases, but there is a lack of CFTR-related research in Chinese CRS children. The most important reason is that CF is considered to be a rare disease in China. However, in recent years, Chinese researchers have found that, due to under-recognition of the disease and limited testing institutions and equipment, there is a significant delay in the diagnosis of CF in China [8]. In other words, CF may not be that rare in China. Therefore, we can speculate that there may be more *CFTR* variants in the Chinese CRS population, and these variants may also play a role in the occurrence and development of CRS. This study is the first time *CFTR* variants have been detected in the Chinese CRS children, and the first in-depth analysis of the variants and the disease.

First of all, in our research, the three variants (MAF < 0.05) with the highest carrying rate of CRS in Chinese children were p.I556V, p. E217G and c.1210-12[T], with no common variation sites in western countries, such as F508del, G542X and N1303K. The variants of CFTR-France Database were gathered from 10 laboratories from all over France; CFTR2 collected the *CFTR* variants of 89,052 CF patients from 27 countries and regions, but only 15 East Asians (all from Japan) were included. Compared with our data, only 6 and 19 sites were included in CFTR2 and CFTR-France respectively, which once again shows the ethnic and regional differences of *CFTR* variation [4,5]. In China, there is no database for CF patients because it is considered to be a rare disease. Therefore, in order to understand the variation of *CFTR* in the population and minimize the ethnic and regional differences , we compared the V. A. F of this study with the gnomAD_genome _EAS database. gnomAD_genome _EAS included gene sequencing data from 9435 healthy people in East Asia, but the geographical distribution and ethnic proportion of the personnel included in the database in East Asia are not clear. Therefore, we chose the Chinese population database provided by Beijing Mygenostics co., LTD, which included the full exon sequencing data of 46,648 healthy Han adults in the Chinese mainland area. However, by comparison, it was found that there were still 2 sites without data. c.1766 + 82dup is rated as VUS, but it is not included in Clinvar, HGMD, CFTR2, CFTR-France, gnomAD_genome _EAS database or Mygenostics database, and is considered to be a new variant. The common pathogenic sites detected in CF-related studies in China are also different from the results of this study.

In the subgroup analysis, we found that the number of *CFTR* variants in the recurrent upper respiratory infection group and FESS group was higher than that in the non-recurrent upper respiratory infection group and adenoidectomy group. However, limited by the sample size, there was no statistical difference in the carrying rate of the sites between the two subgroups. However, by comparing the carrying rate of the recurrent upper respiratory infection group and the FESS group with the Mygenostics database, it was found that the carrying rate of p.I807 M, p.V920L, c.1210-12[T] (5T) was significantly higher. We mark these sites in the domain of CFTR and used different software to predict the harmfulness of *CFTR* variants.

Table 5

Comparison of *CFTR* variants between database and subgroups.

NO.	HGVS.c	V. A. F				Mygenostics Database (n = 46,648)	P-value					
		Recurrent Upper Respiratory Infection Group (n = 79)	non-Recurrent Upper Respiratory Infection Group (n = 27)	FESS Group (n = 51)	Adenoidectomy Group (n = 26)		P1	P2	P3	P4	P5	P6
1	c.-152G>C	0.0063	0	0.0098	0	N/A	/	/	1	/	/	1
2	c.91C>T (p. Arg31Cys; legacy: R31C)	0.0063	0	0.0098	0	0.00458754	0.5169	1	1	0.3749	1	1
3	c.374T>C (p. Ile125Thr; legacy: I125T)	0.0190	0	0.0294	0	0.01064354	0.2380	1	0.5722	0.0960	1	0.5513
4	c.650A>G (p. Glu217Gly; legacy: E217G)	0.0190	0.0556	0.0196	0.0385	0.01269079	0.4576	0.0315	0.1741	0.3722	0.1415	0.6038
5	c.1666A>G (p. Ile556Val; legacy: I556V)	0.0443	0.0185	0.0490	0.0192	0.04537172	1	0.5195	0.6827	0.8097	0.7321	0.6643
6	c.1950C>A (p. Phe650Leu; legacy: F650L)	0.0063	0	0.0098	0	0.00112545	0.1643	1	1	0.1094	1	1
7	c.2421A>G (p. Ile807Met; legacy: I807 M) #	0.0063	0	0.0098	0	0.00008575	**0.0151**	1	1	**0.0098**	1	1
8	c.2758G>T (p. Val920Leu; legacy: V920L) #	0.0063	0	0.0098	0	0.00001072	**0.0034**	1	1	**0.0022**	1	1
9	c.*1043A>C	0.0063	0	0.0098	0	0.00321827	0.4005	1	1	0.2815	1	1
10	c.164 + 16T>C #	0.0063	0	0	0	0.00003216	**0.0067**	1	1	1	1	1
11	c.489 + 91A>G	0.0063	0	0	0.0192	0.00199365	0.2715	1	1	1	0.0990	0.3377
12	c.1210-12[T]	0.0316	0	0.0294	0.0192	0.01140153	**0.0359**	1	0.3322	0.1119	0.4494	1
13	c.1680-113C>T #	0.0253	0	0.0196	0	0.00392948	**0.0038**	1	0.5743	**0.0008**	1	0.3006
14	c.1766 + 82dup	0	0.0185	0	0	N/A	/	/	0.2547	/	/	1
15	c.2620-180G>A	0.0063	0	0.0098	0	0.00295529	0.3749	1	1	0.2617	1	1
16	c.3717 + 45G>A	0.0063	0	0	0	0.00728863	1	1	1	1	1	1
17	c.3717 + 92A>G #	0.0063	0	0.0098	0	0.00001072	**0.0034**	1	1	**0.0022**	1	1
18	c.3718-2476G>A	0.0063	0	0.0098	0	0.00071185	0.1085	1	1	0.0715	1	1
19	c.3874-200G>A #	0.0253	0	0.0098	0	0.00132893	**<0.0001**	1	0.5743	0.1306	1	1

P1: Recurrent Upper Respiratory Infection Group VS Mygenostics Database.

P2: non-Recurrent Upper Respiratory Infection Group VS Mygenostics Database.

P3: Recurrent Upper Respiratory Infection Group VS non-Recurrent Upper Respiratory Infection Group.

P4: FESS Group VS Mygenostics Database.

P5: Adenoidectomy Group VS Mygenostics Database.

P6: FESS Group VS Adenoidectomy Group.

The V.A.F of *CFTR* variants in the subgroup was significantly higher than that in the Mygenostics database, P < 0.05.
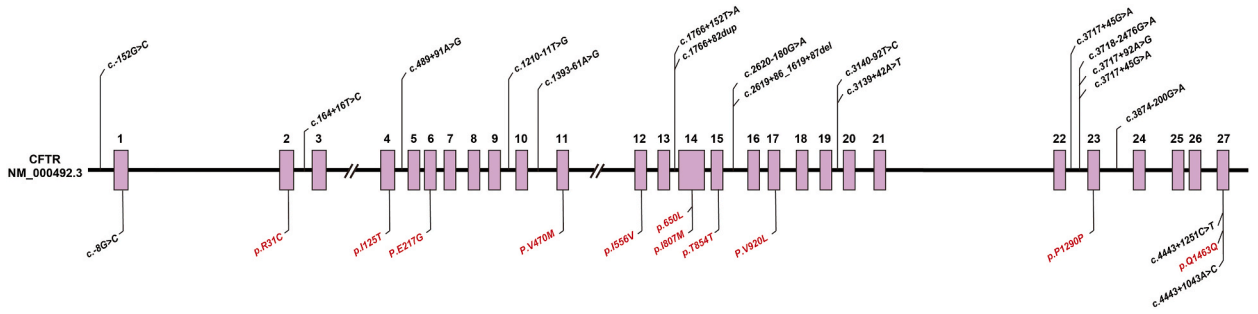
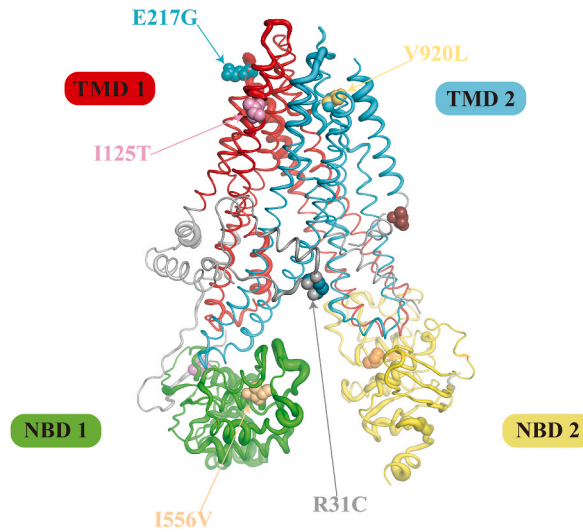**Fig. 2.** CFTR schematic diagram.

Fig. 3. 3D structure of CFTR protein and the position of some variants in the domain.

CFTR is an PKA-activated cAMP-dependent anion channel expressed on the epithelial surface, with two transmembrane domains: TMD1 and TMD2. Each TMD contains 6 transmembrane segments (TM); on the cytoplasmic side, there are two cytosolic nucleotide-binding domains: NBD1 and NBD2, and a unique regulatory domain R [19]. The transmembrane structure is mainly formed by the arrangement of TM1-6 of TMD1 and TM8-12 of TMD2. When the pores open, Cl^- is excreted out of the cells. Therefore, the function of
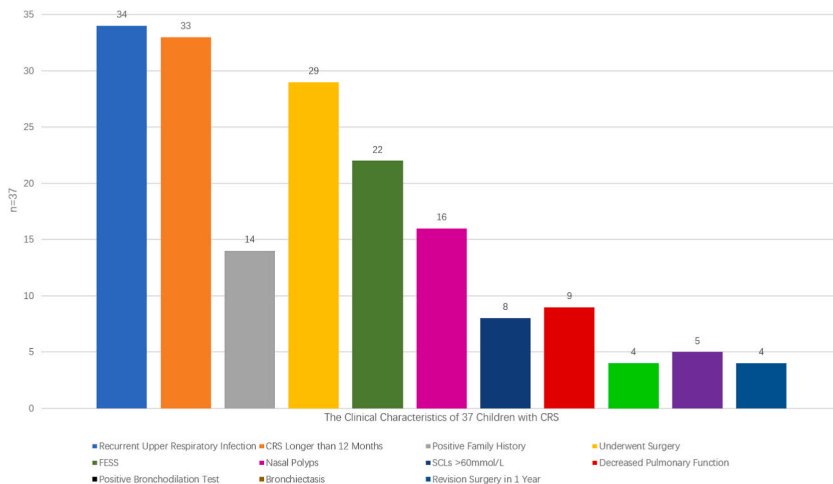
Fig. 4. Clinical characteristics of patients carrying *CFTR* variants (MAF < 0.05).

Table 6
Follow-up analysis of decreased pulmonary function in CRS patients who carrying *CFTR* variants (MAF < 0.05).

NO.		Before Operation								3 Months after Operation							
		FVC	FEV1	FEV1 % FVC	PEF	FEF25	FEF50	FEF75	MMEF 75/25	FVC	FEV1	FEV1 % FVC	PEF	FEF25	FEF50	FEF75	MMEF 75/25
1	**Patient 28**	114.7	106.6	91.8	78.4	76.3	**69.5**	**42.2**	**62.7**	125.1	112.5	**88.8**	88.6	83.3	**67.1**	**45.6**	**61.8**
2	**Patient 31**	96.2	**74.5**	**76.5**	66.9	50.7	41	**36.9**	**40.9**	101.3	83.4	**81.4**	63.6	65.8	51.2	50.3	51.6
3	**Patient 33**	69.2	68	97.1	**59.7**	65.6	56.8	51.8	55.5	106.5	100.2	92.9	85.8	83.4	70.9	**52.5**	66
4	Patient 36	108.2	108.6	101.6	96.3	92	85.2	68.5	81.9	106.4	100.3	95.4	90.8	76.1	74.2	**54.1**	71.4
5	**Patient 40**	104.9	87.7	**82.5**	88.3	63	**46.5**	**36.5**	**44.6**	109.6	106.3	95.7	105.7	97.3	73.2	**56.8**	69.9
6	Patient 48	95.4	88.2	91.3	102	77.5	**54.7**	**28.1**	**45.8**	107	100.1	92.4	91.4	89.6	**69.5**	**45.7**	67.8
7	Patient 52	**80.9**	**76.3**	93.3	82.1	77.7	**57.5**	**37.8**	**52.3**	108.4	103.3	94.1	85.2	96.3	92.2	**60.7**	75.6
8	Patient 54	109.6	98.7	91.2	92	87.4	**58.8**	**47.8**	**59.2**	104.7	95.7	95.7	**73.3**	75.4	**63.8**	**45.7**	62
9	**Patient 55**	104.8	95	**89.5**	98.7	84	**56.5**	**46.5**	**56.2**	104.4	94.3	**89.2**	98.9	**75.9**	58	**47.3**	56.7

A total of 9 patients showed a decrease in pulmonary function, and the decrease of small airway function was more obvious. They underwent pulmonary function examinations again 3 months after the operation, but couldn't recover completely. Patients 28, 31, 33, 40, 55 were diagnosed with bronchiectasis by lung CT. Patients 33, 40, 55 had revision surgery 1 year after the operation.

TMD may be the key to the function of CFTR as anion channel [4]. However, p.E217G (Fig. 3 and 5A and B) is located in the TMD1 domain of CFTR protein, and p.V920L is located in the TMD2 domain. They may reduce Cl⁻ transport by affecting pore size or opening time. Studies have shown that p.E217G mutations reduce membrane protein expression and anion transport activity by 60–70% in East Asian populations [20]. p.V920L, the sequence change replaces valine, which is neutral and non-polar, with leucine, at codon 920 of the CFTR protein (Fig. 5C and D). This amino acid position is highly conserved in available species and the missense change has been observed in individual(s) with CFTR-related conditions [21,22]. However, no experimental evidence demonstrating an impact on protein function has been reported. In this study, Patient 54, which carries both these two variants, was diagnosed as ACP and underwent FESS. As a school-age child, she clearly described the rapid deterioration of nasal symptoms within a month, and the effect of drug treatment was very poor. Fortunately, there was no recurrence of polyps so far.

RD is a unique regulatory domain of CFTR, which lies between two TMD-NBD complexes and contains multiple serine/threonine residues. Studies have shown that RD may evolve from non-coding sequences and is the key regulatory region of CFTR protein: CFTR can be effectively activated only when RD is phosphorylated by PKA [23]. p.I807 M, which the isoleucine at codon 807 is replaced by
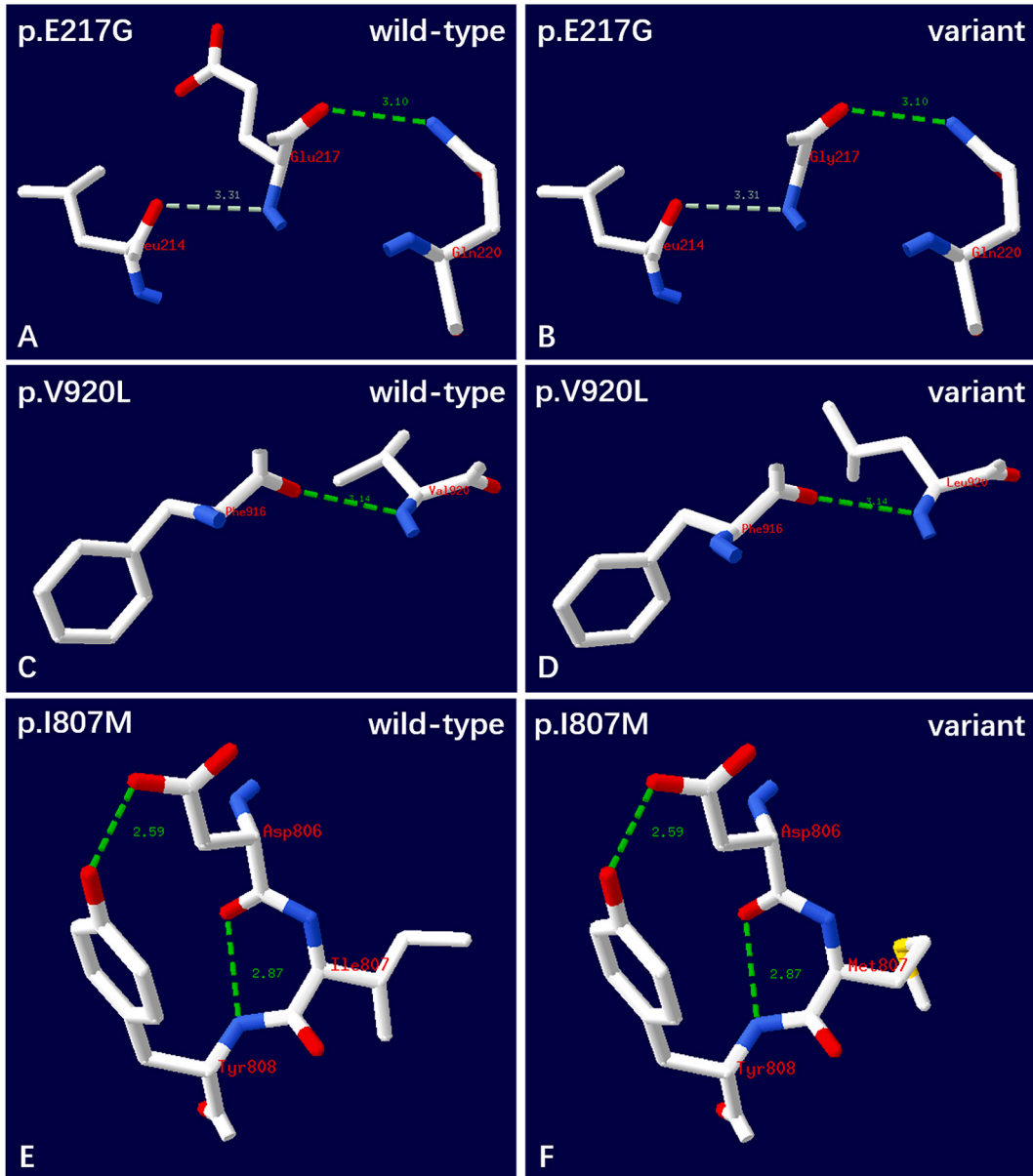

Fig. 5. 3D structure of p.E217G (A, B), p.V920L (C, D) and p.I807M (E, F).
Gray represents non-polar amino acids. Yellow represents an uncharged polar amino acid. Red represents negatively charged amino acid residues. Blue represents positively charged amino acid residues. Green stands for hydrogen bond.

methionine, is located in the RD (Figs. 3 and 5E, F). This amino acid position is highly conserved in available vertebrate species. Consistent with the results of previous studies, SIFT tool predict that this variant is deleterious. However, functional studies show no defects in protein maturation or chloride channel activity [24]. Due to conflicting information, the clinical significance of the p.I807 M variant is uncertain. It has been reported that p.I807 M is related to congenital bilateral absence of the vas deferens (CBAVD) and chronic pancreatitis [18]. Although p.I807 M annotated as "CFTR-RD-causing" in CFTR-France database, the report of p.I807 M in CRS is very rare. In this study, Patient 33, who carried 4 variants (including p.E217G and p.I807 M), suffered from refractory CRS and bronchiectasis. Moreover, even if the variants carried by the patient are only from the mother, the possibility of them impacting the disease can't be dismissed, especially when the patient carries multiple *CFTR* variants (Patients 33, 41, 80, 101). Studies have shown that *CFTR* heterozygotes can also be highly associated with diseases, especially when multiple variants interact and ultimately affect the overall function of CFTR [20].

Although cystic fibrosis of the lungs is the most prominent pathological manifestation of CF, CFTR is widely expressed in the respiratory epithelium, gastrointestinal epithelium, bile duct, pancreas, sweat glands and some genital epithelia. This is also the main reason why CFTR dysfunction can lead to complex disease syndromes. c.1210-12[T] is 5T, which is annotated as "Varying clinical consequence" in CFTR2 and CFTR-France. 5T is associated with many phenotypes, and there isn't enough current clinical experience to accurately predict the risk of this gene variant [25]. However, some studies have shown that 5T variant are more common in CBAVD or pancreatitis than in CF patients [26–28]. In our research, we can observe a significant increase in the carrying rate of 5T in CRS children. Longer TG repeat sizes are associated with a greater susceptibility to disease. In this study, Patient 80 was TG13 (the rest were TG12, Table S7). Although Patient 80 (carrying 2 variants (MAF < 0.05) had no nasal polyps, his CRS showed poor drug efficacy. At the same time, we observed that he suffered from chronic diarrhea. However, his guardian refused further examination, so we were unable to obtain more clinical data such as sweat chlorides. Therefore, future functional studies are required to assess 5T in Chinese CRS children. The effect of 5T on CFTR shows that the non-coding region plays an important role in gene regulation.

CRS is a highly heterogeneous chronic inflammation of nasal mucosa. Although there are reports of familial aggregation, the genetic model of CRS is not clear and the familial segregation is unavailable [29]. In our study, 83.02% of the patients carried more than 2 variants. The clinical phenotypes of children carrying variants (MAF < 0.05) were sorted out and compared, and it was found that all children's variants (MAF < 0.05) were heterozygous. Among them, 91.89% were accompanied by repeated upper respiratory tract infections. In other words, children having these variants does not affect the quantity or function of CFTR proteins in the body under normal circumstances. However, in the case of external environmental changes such as smoking/exposure to secondhand smoke, air pollution, exposure to allergens, or microbial invasion such as viruses, the anti-strike ability and resilience of CFTR may be weakened [30]. This may be one of the reasons for the increase in the frequency of upper respiratory infections in children. However, upper respiratory infection is one of the most important causes of CRS in children [14], which means, carrying multiple *CFTR* variants, especially p.E217G, p.I807 M, p.V920L and c.1210-12[T] may increase susceptibility to CRS. Therefore, in the work of pediatric-otolaryngologists, we shouldn't only pay attention to the carrying of *CFTR* variants in children with CRS, but we also need to evaluate the overall airway function of patients, and not just focus on the treatment of CRS.

CFTR-related disorder is a monosymptomatic clinical entity associated with CFTR dysfunction that does not fulfill the diagnostic criteria for CF [31]. Patient 41 is characterized by recurrent upper respiratory infection and highly active and proliferative nasal polyps, but there is no obvious pulmonary function decrease. The two variants he carried were 5T and p.I556V, from his father and mother respectively, both of which were associated with CFTR dysfunction [18]. However, the SCLs in this child was lower than 60 mmol/L. In fact, the levels of sweat chloride levels in patients are not always consistent with the results of genetic verification in our results. In contrast to Patient 41, 8 patients in this study had SCLs >60 mmol/L, but their parents' generation verification tests suggested that the variants only originated from their father or mother, so the diagnosis of CF was not supported. Some studies have shown that the SCLs lacks sensitivity and specificity in identifying CFTR dysfunction with non-classical manifestations [32,33]. This may be one of the reasons for the inconsistency between the results of SCLs and genetic verification. Danieli et al. have shown that 38% of those ultimately diagnosed with CF had initial SC between 20 and 30 mmol/L, and then their sweat chloride levels increase with age and disease progression [34]. This suggests that there are CFTR-related disorders in patients with CRS. It can even be speculated that there may be mild CF in patients with low age, long-term CRS, or recurrent upper respiratory infections.

There are some limitations in this study. First of all, this is a single-center study. The number of samples is insufficient, and there is no open or large sample size of the Chinese population's genetic data, and there is no database for *CFTR* variants. There are few studies on CFTR-related disorders in China, and most of the patients' clinical manifestations are atypical. However, due to the limited medical resources, it is not possible to conduct a comprehensive examination of every child with CRS, especially the patients with CRSsNP. Currently, the number of people under the age of 14 in China is close to 300 million. Due to the large number of patients in outpatient facilities, pediatric-otolaryngologists often give diagnoses according to their medical history and clinical manifestations. Clinical testing such as fractional concentration of nasal exhaled nitric oxide and lung function are not used frequently. In recent years, allergen detection and serum IgE are becoming more and more popular in clinic, but these tests are mostly used in patients with CRSwNP. Therefore, when reviewing these medical records, the lack of a large amount of clinical data directly leads to the unreliability of some statistical values. On the one hand, in China, the awareness of CFTR dysfunctional diseases is insufficient, and the testing institutions and equipment are also limited, resulting in low SCLs and parents' first-generation verification rate. On the other hand, people are more willing to examine the clinical symptoms that have already appeared. For "underlying diseases" with no obvious clinical manifestations, such as absence of vas deferens, the child's guardian is only willing to provide "deny of medical history" and refuse reproductive system ultrasound examinations. In other words, there may be CFTR-related disorders involving other organs in our study.

5. Conclusion

We observed the *CFTR* variant spectrum in the Chinese population and reported a new variant: c.1766 + 82dup, which expanded the known gene variant spectrum, although this is not pathogenic based on the prediction by Splice AI. The carrying rate of *CFTR* variants in Chinese CRS children increased, and the highest rates of variants (MAF < 0.05) are p.I556V, p. E217G, c.1210-12[T]. Carrying multiple *CFTR* variants, especially p.E217G, p.I807M, p.V920L and c.1210-12[T] may lead to increased susceptibility to CRS. There are also CF-related disorders in patients with CRS. Therefore, CRS children who cannot achieve good results after treatment need to be tested for *CFTR* variants or have more examinations for CFTR-related disorders to understand the potential possibility of CF.

Informed consent statement

In this study, we obtained the written informed consent statement of patients and the parents or guardians of them. They all agreed to provide the biological samples for research and that the patient's clinical data could be collected and analyzed. All informed consent is recorded in the《Informed Consent Form》with patients and/or parents and guardians.

Consent for publication

All authors listed have read the complete manuscript and have approved submission of the paper.

Data availability statement

We declare that submitted manuscript does not contain previously published material and is not under consideration for publication elsewhere. The manuscript is a truthful and original work without fabrication, fraud or plagiarism. All the data included in article/supp, material/referenced in article.

CRediT authorship contribution statement

Yang Han: Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Jinhao Zhao:** Formal analysis, Data curation. **Wenjing Liu:** Formal analysis, Data curation. **Xiaojian Yang:** Software, Methodology. **Wei Zhang:** Formal analysis, Data curation. **Xiao Xiao:** Formal analysis, Data curation. **Xiaoge Liu:** Software, Methodology, Investigation. **Xiaoxu Chen:** Software, Formal analysis, Data curation. **Lixing Tang:** Software, Methodology. **Pengpeng Wang:** Writing – review & editing, Supervision, Resources. **Wentong Ge:** Writing – review & editing, Visualization, Supervision, Resources, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

List of Abbreviation

CRS	Chronic Rhinosinusitis
CFTR	Cystic Fibrosis Transmembrane Conductance regulator
CF	Cystic Fibrosis
FESS	Functional Endoscopic Sinus Surgery
WES	Whole Exome Sequencing
MLPA	Multiplex Ligation-dependent Probe Amplification
ACP	Antrochoanal Polyp;
CRSwNP	Chronic Sinusitis with Nasal Polyp;
SCLs	Sweat Chloride Levels
ACMG	American College of Medical Genetics and Genomics

VUS   Variant of Uncertain Significance
HGMD   The Human Gene Mutation Database
DM   Disease-causing Mutation
DM?   likely Disease-causing Mutation
FP   in vitro or in vivo Functional Polymorphism
DFP   Disease-associated Polymorphism with additional Functional Evidence
MAF   Minor Allele Frequency
SNP   Single Nucleotide Polymorphism
CBAVD   Congenital Bilateral Absence of the Vas Deferens

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e27681.

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