OPEN ACCESS

Original research

Genetic spectrum of Chinese children with cystic fibrosis: comprehensive data analysis from the main referral centre in China

Yuelin Shen,[1] Xiaolei Tang,[1] Qionghua Chen,[2] Hui Xu,[1] Hui Liu,[1] Jinrong Liu,[1] Haiming Yang,[1] Huimin Li,[1] Shunying Zhao [1]

► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/jmg-2022-108501).

[1]Department II of Respiratory Medicine, National Clinical Research Center for Respiratory Diseases, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, Beijing, People's Republic of China
[2]Quanzhou Children's Hospital, Quanzhou, People's Republic of China

Correspondence to
Professor Shunying Zhao, Beijing Children's Hospital Capital Medical University, Beijing 100045, China; shunyingzhao@yeah.net

Received 16 February 2022
Accepted 3 July 2022
Published Online First 20 July 2022

ABSTRACT

Background and objectives Cystic fibrosis (CF) is a heterogeneous disease with a diverse genetic spectrum among populations. Few patients with CF of Chinese origin have been reported worldwide. The objective of this study is to characterise the genotypic features of CF in Chinese children.

Methods We recruited and characterised the genetic manifestations of 103 Chinese children with CF in Beijing Children's Hospital from 2010 to 2022. Whole-exome sequencing were performed to define the genotypes. Meanwhile, other 99 genetically confirmed patients with Chinese origin described in 45 references were also summarised.

Results 158 different variants including 23 novel observations were identified after sequencing. The majority of *CFTR* variants (82.3%) in Chinese have been observed only once or twice. 43.7% of the variants were only identified in patients of Chinese origin. The c.2909G>A(p.Gly970Asp), c.1766+5G>T and c.1657C>T(p.Arg553X) were the most frequent variants among Chinese patients, with allele frequency of 12.1%, 5.4% and 3.6%, respectively. The first two variants both showed significant Chinese ethnic tendency, while the latter one most likely came from Europeans for historical reasons. They also demonstrated significant differences in geographical distribution. c.1521_1523delCTT(p.F508del) was rarely observed in patients of pure Chinese origin, with an allele frequency of 1.8%. Two de novo variants (c.960dupA[p.Ser321IlefsX43] and c.2491-2A>G) and two deep-intronic variants (c.3718–2477C>T and c.3874-4522A>G) were identified, which were also quite rare among Chinese.

Conclusions The genetic spectrum of CF in Chinese is unique and quite different from that observed in Caucasians. The geographical distributions of the most frequent variants were reported for the first time.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Cystic fibrosis (CF) is a rare disease in Chinese populations, with only approximately 110 CF patients of Chinese origin were reported in literature up to now. Furthermore, most of publications were case reports.

WHAT THIS STUDY ADDS

⇒ This is the largest study and most comprehensive analysis of genotypic features of CF in Chinese population to date. The genetic spectrum of CF in Chinese is unique and quite different from that observed in Caucasians. Meanwhile, the geographical distributions of the most frequent variants were reported for the first time in this study.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This study could expand our knowledge on the genotypic features of CF in Chinese population, which will greatly contribute to prevention, diagnosis and even future molecular genetic management of the disease in China.

Cystic fibrosis (CF) is the most frequent monogenic disease in Caucasian populations, with incidences ranging from 1/1800 to 1/25000.[1 2] According to the American Cystic Fibrosis Foundation registry, there are currently more than 100 000 CF patients throughout the world. China is the world's most populated country with a population of approximately 1.4 billion. However, up to now, only approximately 110 CF patients of Chinese origin were reported in literature,[3] and most of publications were case reports, without any epidemiological data on the prevalence available. Interestingly, most of the cases have been diagnosed in the last 5 years, which suggests that the actual incidence of CF in China may be seriously underestimated. The contributing factors may include underdiagnosis, under-reporting, lack of national registries and variability in the frequency of mutation carriers. It has been identified that the profiles of CF transmembrane conductance regulator (CFTR) gene mutation spectrum vary widely among different populations based on their geographic and ethnic origins. Although CF in China is being increasingly recognised, there is still an urgent need to understand the genetic spectrum of the *CFTR* gene in Chinese patients, which will greatly contribute to prevention, diagnosis and even future molecular genetic management of the disease.

In this study, we describe 103 children with CF receiving care at the main referral centre in China. Meanwhile, other 99 genetically confirmed cases with Chinese origin reported worldwide from 1993 to 2022 were also summarised. To our knowledge, this is the largest study and most comprehensive

To cite: Shen Y, Tang X, Chen Q, *et al*. *J Med Genet* 2023;**60**:310–315.

analysis of genotypic features of CF in Chinese population to date.

STUDY DESIGN AND METHODS

Children referred to the Respiratory Department of Beijing Children's Hospital from January 2010 to May 2022 were enrolled in the study after meeting the diagnostic criteria of CF. CF was diagnosed based on the Consensus Guidelines from the Cystic Fibrosis Foundation[2]: at least one of the key clinical features highly suggestive of CF, including sinopulmonary, gastrointestinal, reproductive systems manifestations, as well as evidence of *CFTR* dysfunction, including elevated sweat test and/or presence of biallelic pathogenic variants.[2] Duplicate cases that had been reported in previous literatures were excluded.

In addition, we studied all the published CF cases of Chinese origin by searching China National Knowledge Infrastructure database, Wanfang database, PubMed, Embase, Cochrane Library, OVID medicine and SinoMed databases from January 1975 to January 2022. The search strategy included the following term keys: ('cystic fibrosis') AND ('Chinese' OR 'China'). Study types included clinical trials, meta-analyses, randomised controlled trials, case reports, case series or reviews. Original articles were included if they met the criteria including the patients were of Chinese origin, the presence of CF disease and complete data of both clinical manifestations and genetic sequencing. Incomplete cases or duplicate reports were excluded from our final analysis.

SWEAT CONDUCTIVITY MEASUREMENT

The Macroduct collection system and Sweat-Chek conductivity analyzer (Wescor Inc, Logan, Utah, USA) were used for CF sweat conductivity analysis as previously described.[4–6] Based on a user's manual, sweat secretion was stimulated by pilocarpine iontophoresis. Following the stimulation, sweat was collected in a coiled plastic tubing collector cup for 30 min. Then, the sweat sample was transferred from the Macroduct tube to the take-up tube on the conductivity cell during the analysis. The test was repeated on two separate days. The average results were considered normal if values were below 60 mmol/L and intermediate if values were between 60 and 80 mmol/L.[7] CF was very likely if values were equal to or above 80 mmol/L.

CFTR gene sequencing

Genomic DNA samples were extracted from peripheral blood leukocytes by using standard genomic DNA purification methods. The whole-exome sequencing, bioinformatics analysis and Sanger sequencing validation were performed according to their standard approach as previously described.[8] Multiplex ligation-dependent probe amplification (MLPA) (MRC-Holland) was applied to detect the large deletions or duplications of CFTR gene. All exons, proximal introns and selected regions of deep introns were fully analysed.

RESULTS

Demographic data

A total of 103 patients (40 males and 63 females) from 100 Chinese families presented to Beijing Children's Hospital were recruited into this study. All 103 patients were of Chinese origin, and none of them had a family history of intermarriage with Caucasians. Patients no. 30-1/no. 30-2, no. 31-1/no.31-2 and No. 76-1/no.7622 were siblings respectively both suffering from CF. Only two children (case no. 30-1/no. 30-2) from the same family were the products of consanguineous marriage. The mean (±SD) ages at CF diagnosis were 7.6 (±4.4) years. Eighty-four children accepted sweat conductivity analysis; among them, 78 children had positive results and six of them showed intermediate values. The mean value was 114.1 mmol/L ranging from 60.0 to 168.0 mmol/L (online supplemental e-table 1).

In addition, other 99 Chinese patients from 94 different families described in 45 references were included in this study (Mainland China (82 cases), [4 6 9–41] Taiwan area (nine cases),[42–47] Hong Kong (five cases),[48] Australia (one case),[49] Canada (one case)[50] and USA (one case)).[51] Overall, a total of 202 CF patients of Chinese origin constituted the cohort for the current analysis.

Genetic spectrum summarising

The *CFTR* gene variants identified in all the 103 patients (100 families) presented to Beijing Children's Hospital are listed in online supplemental e-table 1. Of note, cases 1~19[4] and cases 20~31[6] (except for patient no. 30-1 and no. 31-1) have been reported in 2016 and 2020, respectively, by the authors. The remaining cases have never been reported so far. Twenty-three novel observations were identified after sequencing (c.222delG[p.Arg75AspfsX16], c.298C>T[p.Leu100Phe], c.464C>G[p.Ala155Gly], c.940G>T[p.Gly314Trp], c.1064C>G[p.Pro355Arg], c.1219G>T[p.Glu407X], c.1265C>T[p.Ser422Phe], c.1347_1350delAGAA[p.Arg450AspfsX18], c.1368delT[p.Ala457LeufsX12], c.1393-?_1584+?del, c.1514delA[p.Asn505IlefsX22], c.1523_1534delTTGGTGTTTCCT[p.Phe508_Ser511del], c.1772T>C[p.Val591Ala], c.1810A>C[p.Thr604Pro], c.2042A>T[p.Glu681Val], c.2058_2061delTT [p.Phe687X], c.2328dupA[p.Val777SerfsX2], c.2489dupA[p.Glu831GlyfsX5], c.2909-?_3468+?del, c.3140-?_3367+?del, c.3469-12T>G, c.3469-2A>T and c.3659C>T[p.Thr1220Ile]).

The spectrum of *CFTR* variants detected in all Chinese patients with CF (194 families, 388 alleles) was summarised in online supplemental e-table 2. As a result, 373 mutated alleles were detected (detection rate: 96.1%). The identified variants included 56 missense, 31 nonsense, 27 frameshift, 21 splicing, 11 large insertion/deletion, 8 sequence variation and 4 in-frame insertion/deletion. Overall, 158 different variants were identified after sequencing. The majority of *CFTR* variants (82.3%, 130/158) in Chinese have been observed only once or twice. Approximately half of the variants (43.7%, 69/158) were only identified in patients of Chinese origin thus far, and to our knowledge, they have never been reported in Caucasians (online supplemental e-table 2). The c.2909G>A(p.Gly970Asp) was found to be the most frequent variant among Chinese CF patients with the highest allele frequency of 12.1% (47/388), followed by c.1766+5G>T (5.4% (21/388)) and c.1657C>T[p.Arg553X] (3.6% (14/388)), which were the second and third most common variants, respectively (online supplemental e-table 2, figure 1). They also showed significant differences in geographical distribution, that is, the c.2909G>A(p.Gly970Asp) variant was found in the Northern and Eastern China, while the c.1766+5G>T and the c.1657C>T(p.Arg553X) variants were most common observed in the Southern and Eastern coasts (figure 2). Meanwhile, c.1521_1523delCTT(p.F508del) was observed in six patients of pure Chinese origin, with an allele frequency of 1.8% (7/388). Interestingly, two de novo variants (c.960dupA[p.Ser321IlefsX43] and c.2491-2A>G) and two deep-intronic variants (c.3718-2477C>T and c.3874-4522A>G) were identified, which were also quite rare among Chinese.

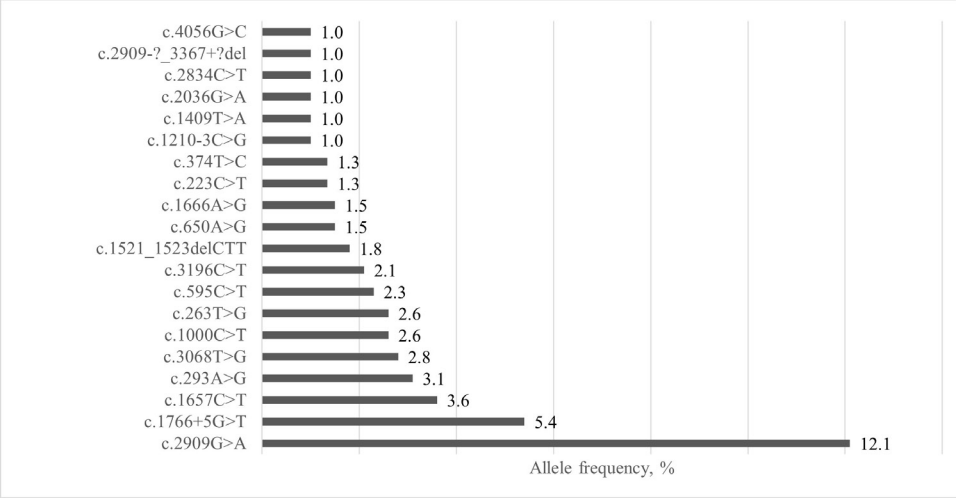

Figure 1 Frequency (>1%) of CFTR variants observed among 202 Chinese CF patients. CF, cystic fibrosis; CFTR, CF transmembrane conductance regulator.

DISCUSSION

Herein, to the best of our knowledge, we report results of the most comprehensive analysis to date of genotypic features associated with CF patients originating from across China. The first CF patient of Chinese origin was reported in 1975, which was diagnosed by sweat test.[52] Then, it was not until 1993 that there was the first genetically confirmed case.[46] From 1993 to 2022, a total of 202 CF patients of Chinese origin have been diagnosed with definite *CFTR* variants. In addition, 158 different variants of *CFTR* gene were identified, including 23 novel observations (current report). Of these, only 45 variants are known to be CF causing in CFTR2.[53] In addition, 50 loss-of-function variants (nonsense, frameshift and large insertion/deletion) are likely to be CF causing. However, the pathogenic significance for the remaining 63 variants is unknown, and further functional elucidation is necessary.

The variant spectrum of *CFTR* among Caucasians in Western countries has been well established. c.1521_1523delCTT(p.F508del) is the most frequent variant in Caucasians, accounting for approximately 70% of mutated alleles in general.[54] However, it is quite rarely seen in Asia, especially East Asia.[55] The c.1521_1523delCTT (p.F508del) was only observed in six patients of Chinese origin (one in homozygosity and five in compound heterozygosity), with an allele frequency of only 1.8%. No cases have ever been reported in other East Asian countries so far (except for mixed Asian-Caucasian parentage). By contrast, among Chinese population, the majority of *CFTR* variants (82.3%, 130/158) have been observed only once or twice. Approximately half of the variants (43.7%, 69/158) were only identified in patients of Chinese origin thus far. The c.2909G>A(p.Gly970Asp) and c.1766+5G>T variants were the most predominant observations, occurring in 12.1% and 5.4% of the alleles among all the reported Chinese patients, respectively. Notably, both variants show significant Chinese ethnic tendency, because to our knowledge, most cases with them were reported among Chinese. The c.1657C>T(p.Arg553X) was the

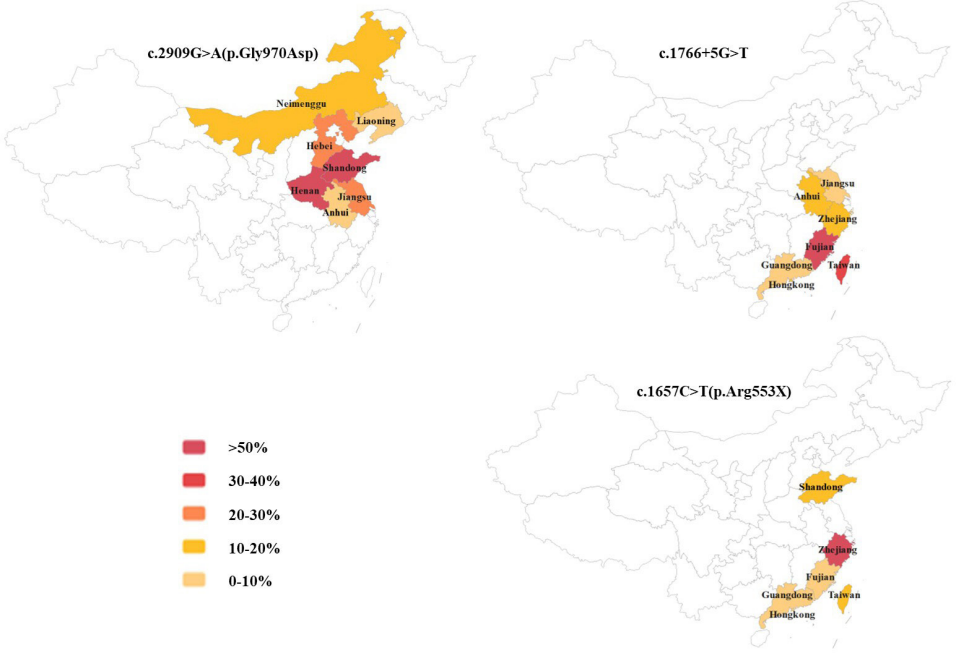

Figure 2 Frequency distribution of the top three most frequent variants among 202 Chinese patients with CF. CF, cystic fibrosis.

third most commonly observed variants, with an allele frequency of 3.6%. Interestingly, the c.1657C>T(p.Arg553X) was also present in the panel of 23 mutations proposed by the American College of Medical Genetics and Genomics.[56] It has been associated with Central European-derived populations, and the clinical consequence of this variant is known to be CF causing.[57] In terms of geographic distribution, the c.2909G>A(p.Gly970Asp) variant was found in the Northern and Eastern China, while the c.1766+5G>T and the c.1657C>T(p.Arg553X) variants were most common observed in the Southern and Eastern coasts (figure 2). We presume that the different distribution of these variants may be due to the different ethnic groups in different parts of China. Eastern coast is considered the most prosperous region of China's economy and trade, with more frequent population migrations from both Northern and Southern areas. That may be a possible explanation that the top three most frequent variants were all found in the Eastern area. The first description of c.1657C>T(p.Arg553X) among Chinese was made in homozygosity genotype in a native Taiwanese boy diagnosed with CF. Chen *et al*[45] proposed that the occurrence of c.1657C>T(p.Arg553X) variant in Taiwan area may correspond to the colonisation by the Dutch and Spanish 300 years ago. Interestingly, in this study, half of the c.1657C>T(p.Arg553X) variants were found in Zhejiang Province, which located on the southeastern coast of China. Historically, since China established diplomatic relations with European countries in the 1950s, Zhejiang people immigrated to Europe through Macao and Hong Kong. At present, Zhejiang migrants live throughout Europe. Based on this, we speculate that the origin of the c.1657C>T(p.Arg553X) variant most likely came from Europeans, which may be due to the intermarriage between Taiwanese and Europeans during the colonial period, or the wave of European economic immigration after the founding of new China.

Variants occurring de novo in the *CFTR* gene are extremely rare, with approximately 10 cases of de novo CFTR variants published to date.[58] There was an interesting finding that two de novo variants (c.960dupA[p.Ser321IlefsX43] and c.2491-2A>G) were identified among Chinese, which were confirmed after paternity test as well as *CFTR* gene screening for the biological parents. Furthermore, both de novo variants were found in Beijing Children's Hospital. The insertion c.960dupA(p.Ser321IlefsX43) has been reported in 2016,[4] while the splicing variant c.2491-2A>G was a recent observation. Among previous reports, the only description of the c.2491-2A>G was made in an Irish CF patient, without any additional clinical data available.[59] In the present study, c.2491-2A>G was the first time observed as a de novo variant in a 11-year-old Chinese boy (case no. 68) with severe sinopulmonary manifestations, liver disease and pancreatic insufficiency (PI), who bore the c.2491-2A>G/c.3196C>T genotype disease. Compared with inherited variants, de novo variants are probably more deleterious because they have been subjected to less stringent evolutionary selection.[60] Most often, the de novo variants appeared on the paternal chromosome, and the same observation was also shown for c.24912A>G in our patient. Casals *et al*[61] proposed that this tendency may reflect a higher mutation rate in paternal gametes. Nevertheless, the c.960dupA (p.Ser321IlefsX43) earlier found in our study was located on the maternal chromosome. The reason for this is unknown.

The *CFTR* genotype remains incomplete in 1% of CF cases, deep-intronic variants are putative candidates to fill this gap.[62] A collection of variants in non-coding regions of the *CFTR* gene could help to assess their potential role as genetic factors that modify the phenotype. To our knowledge, only seven deep-intronic disease-causing variants have been identified in the *CFTR* gene until now.[62] Interestingly, two deep-intronic variants (c.3718-2477C>T[c.3717+12 191C>T] and c.3874-4522A>G) were the first time identified among Chinese patients. The c.3718-2477C>T has been associated with multiethnicity-derived populations, including Ashkenazi-Jewish, Southern European, Middle Eastern, Iranian and Indian.[57] In addition, it belongs to class V mutations in the CFTR2 database, which result in reduced synthesis of CFTR protein with normal function at the epithelial cell membrane.[57 63] In our report, the c.3718-2477C>T variant was found in two Chinese CF children (case no. 44 and case no. 83) with typical pulmonary features but without PI. The results of sweat conductivity test were intermediate (64 mmol/L) and weak positive (87 mmol/L), respectively, which were consistent with previous reports and may be considered milder CF phenotype. As far as c.3874-4522A>G was concerned, the ethnic origins were reported to be France, Iran and Laos.[62] Moreover, this variant was considered CF causing associated with a large phenotypic spectrum, including CFTR-related disorders and typical CF.[62] In the present study, c.3874-4522A>G was found in two Chinese children (case no. 39 and case no. 57) with severe sinopulmonary diseases from early childhood, including progressive bronchiectasis, recurrent airway *Pseudomonas aeruginosa*, lung function defect and sinusitis (both cases), and allergic bronchopulmonary aspergillosis (only for case no. 39). Due to the limited sequencing methods at the first time they presented to us, we only detected the c.2936A>C(p.Asp979Ala) and the c.1368delT(p.Ala457LeufsX12) variant on one allele, respectively. Three years later, we found the c.3874-4522A>G variant deeply located in intron 23 of the other allele and eventually supplemented the genetic data for both children.

In terms of the novel observations, 12 variants (c.222delG[p.Arg75AspfsX16], c.1219G>T[p.Glu407X], c.1347_1350delAGAA[p.Arg450AspfsX18], c.1368delT[p.Ala457LeufsX12], c.1393-?_1584+?del, c.1514delA[p.Asn505IlefsX22], c.1523_1534delTTGGTGTTTCCT[p.Phe508_1521Ser511del], c.2058_2061delTT[p.Phe687X], c.2328dupA[p.Val777SerfsX2], c.2489dupA[p.Glu831GlyfsX5], c.2909-?_3468+?del and c.3140-?_3367+?del) are likely to be CF causing, and the clinical consequences of remaining 11 variants are uncertain. Clinical characterisation includes the presence of high frequency of sinopulmonary diseases and relatively low frequency of PI compared with Caucasians, which were consistent with previous reports on phenotype in individuals with CF of Chinese origin (online supplemental e-table 3). The geographical distribution of the novel variants showed no significant discrepancies between North and South in China.

The genetic spectrum of CF in Chinese is unique and quite different from that observed in Caucasians. Therefore, the Caucasian *CFTR* common mutation-screening panel is not applicable for Chinese patients. We recommend using the extensive *CFTR* gene sequencing (including all *CFTR* exons, their intronic boundaries and selected regions of deep introns) followed by MLPA analysis for effective diagnosis, although it is relatively expensive. If patients with CF are still incompletely genotyped, whole genome sequencing for the identification of unknown deep-intronic variants is advocated.

To date, 2110 variants of *CFTR* have been identified worldwide,[64] but the disease liability of only 401 variants has been ascertained in CFTR2.[53] Based on the nature of the molecular defects in CFTR, small molecules (CFTR modulators) can successfully restore activity to the mutant protein, thereby ameliorating disease manifestations.[55] Unfortunately, few studies on the molecular consequences of Chinese-specific *CFTR* variants have

been reported. The most frequent *CFTR* mutation in Chinese, c.2909G>A(p.Gly970Asp), was predicted to be a gating mutation with partial trafficking defect.[65] Furthermore, lumacaftor/ivacaftor therapy was proven efficacy in both ex vivo[65] and in vivo[66] studies of a patient with c.1521_1523delCTT(p.F508del)/c.2909G>A(p.Gly970Asp) genotype. These findings have significant implications for CFTR modulator use in or availability to the Chinese population. Future studies on functional consequences analysis of Chinese ethnicity-specific variants would be beneficial to take the first step to research on CFTR modulator therapies in China.

The limitation of the present study would be that the sweat chloride concentration test, which has been the gold standard for diagnosis of CF, was not available in China. However, the sweat conductivity measurement has been shown to have excellent correlation with the sweat chloride concentration.[67 68] It is accurate, simple to perform and economical. Thus, the sweat conductivity measurement is used as an assistant diagnostic test for CF in China. As the patients in this study were enrolled over a period of decades, this could have cause unintended bias such as improvement in laboratory technology and lifestyle changes. First, sweat conductivity analysis is not readily available in all the paediatric centres in China, except Beijing Children's Hospital, which is considered the main referral centre for patients with CF from all over the country. Besides this, even in our centre, sweat conductivity analysis was not performed on all the patients enrolled because the testing facility was only available from 2014. Finally, MLPA analysis was only adopted in patients recruited from 2016 onwards also due to inaccessibility of this technique.

In conclusion, results presented herein describing the genetic spectrum of CF in Chinese is unique and quite different from that observed in Caucasians, consistent with our previous statement.[4] The c.2909G>A(p.Gly970Asp), c.1766+5G>T and c.1657C>T(p.Arg553X) are the most frequent variants among Chinese CF patients studied. The geographical distributions of the most frequent variants were reported for the first time. These data demonstrate that it is very urgent and necessary to establish a national CF registry in China, which would be beneficial to compare genetic data intranationally and internationally from an epidemiological perspective, to evaluate the phenotype–genotype association in China and to enhance our understanding of CF pathogenic processes for improvement of disease management and prognosis.

Acknowledgements The authors would like to thank all the patients and their families who participated in this study and all the physicians for their help in accomplishing this work. In addition, YS would like to thank Mr Shanming Xuan for the decades of support, encouragement and care.

Contributors YS: conceptualised and designed the study, validated genetic studies, analysed the data and drafted the initial manuscript. XT and QC: validated genetic studies, carried out the initial analyses, and reviewed and revised the manuscript. HX and HLiu: recruited and evaluated patients, and reviewed and revised the manuscript. JL and HY: designed the data collection instruments, coordinated and supervised data collection, interpreted the results, and reviewed and revised the manuscript. HLi and SZ: conceptualised and designed the study, interpreted the results and critically reviewed the manuscript for important intellectual content. SZ: responsible for the overall content as guarantor. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Funding This work was supported by the National Natural Science Foundation of China (81600002).

Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval This study protocol was approved by the Ethics Committees of Beijing Children's Hospital, China (Approval no. (2022)-E-029-R). Informed written consent was obtained from all participants or parent/legal guardians. Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data relevant to the study are included in the article or uploaded as supplementary information.

ORCID iD

Shunying Zhao http://orcid.org/0000-0003-0843-5968

REFERENCES

1 Colombo C, Littlewood J. The implementation of standards of care in Europe: state of the art. *J Cyst Fibros* 2011;10 Suppl 2:S7–15.
2 Farrell PM, White TB, Ren CL, Hempstead SE, Accurso F, Derichs N, Howenstine M, McColley SA, Rock M, Rosenfeld M, Sermet-Gaudelus I, Southern KW, Marshall BC, Sosnay PR. Diagnosis of cystic fibrosis: consensus guidelines from the cystic fibrosis Foundation. *J Pediatr* 2017;181S:S4–15.
3 Shi R, Wang X, Lu X, Zhu Z, Xu Q, Wang H, Song L, Zhu C. A systematic review of the clinical and genetic characteristics of Chinese patients with cystic fibrosis. *Pediatr Pulmonol* 2020;55:3005–11.
4 Shen Y, Liu J, Zhong L, Mogayzel PJ, Zeitlin PL, Sosnay PR, Zhao S. Clinical phenotypes and genotypic spectrum of cystic fibrosis in Chinese children. *J Pediatr* 2016;171:269–76.
5 Hammond KB, Turcios NL, Gibson LE. Clinical evaluation of the macroduct sweat collection system and conductivity analyzer in the diagnosis of cystic fibrosis. *J Pediatr* 1994;124:255–60.
6 Shen Y, Tang X, Liu J, Li H, Zhao S. Pseudo-Bartter syndrome in Chinese children with cystic fibrosis: clinical features and genotypic findings. *Pediatr Pulmonol* 2020;55:3021–9.
7 Sosnay PR, Siklosi KR, Van Goor F, Kaniecki K, Yu H, Sharma N, Ramalho AS, Amaral MD, Dorfman R, Zielenski J, Masica DL, Karchin R, Millen L, Thomas PJ, Patrinos GP, Corey M, Lewis MH, Rommens JM, Castellani C, Penland CM, Cutting GR. Defining the disease liability of variants in the cystic fibrosis transmembrane conductance regulator gene. *Nat Genet* 2013;45:1160–7.
8 Guan Y, Yang H, Yao X, Xu H, Liu H, Tang X, Hao C, Zhang X, Zhao S, Ge W, Ni X. Clinical and genetic spectrum of children with primary ciliary dyskinesia in China. *Chest* 2021;159:1768–81.
9 Li H, Lin L, Hu X, Li C, Zhang H. Liver failure in a Chinese cystic fibrosis child with homozygous R553X mutation. *Front Pediatr* 2019;7.
10 Liu K, Xu W, Xiao M, Zhao X, Bian C, Zhang Q, Song J, Chen K, Tian X, Liu Y, Xu K-F, Zhang X. Characterization of clinical and genetic spectrum of Chinese patients with cystic fibrosis. *Orphanet J Rare Dis* 2020;15:150.
11 Xie Y, Huang X, Liang Y, Xu L, Pei Y, Cheng Y, Zhang L, Tang W. A new compound heterozygous CFTR mutation in a Chinese family with cystic fibrosis. *Clin Respir J* 2017;11:696–702.
12 Liu Y, Wang L, Tian X, Xu K-F, Xu W, Li X, Yue C, Zhang P, Xiao Y, Zhang X. Characterization of gene mutations and phenotypes of cystic fibrosis in Chinese patients. *Respirology* 2015;20:312–8.
13 Li ZC, Bao YM, Chen JH. [A new compound heterozygous CFTR mutation in a child with cystic fibrosis]. *Zhonghua Er Ke Za Zhi* 2018;56:635–6.
14 Chen B, Zhang S, Yang Y. The first case of CF in mainland China identified by DNA analysis. *Chin J Med Cent* 1995;12:5–9.
15 Li N, Pei P, Bu D-fang, He B, Wang G-fa. A novel CFTR mutation found in a Chinese patient with cystic fibrosis. *Chin Med J* 2006;119:103–9.

16 Zhao X, Liu K, Xu W, Xiao M, Zhang Q, Song J, Chen K, Liu Y, Tian X, Xu K-F, Zhang X. Novel mutation c.1210-3C > G in cis with a poly-T tract of 5T affects CFTR mRNA splicing in a Chinese patient with cystic fibrosis. *Front Med* 2022;16:150–5.
17 Cheng Y, Ning G, Song B, Guo Y-kun, Li X-sheng. A Chinese girl with cystic fibrosis: a case report identified by sweat and genetic tests. *Chin Med J* 2012;125:719.
18 Li L, Wang NL, Gong JY, Wang JS. [Infantile cholestasis caused by CFTR mutation: case report and literature review]. *Zhonghua Er Ke Za Zhi* 2016;54:851–5.
19 Xu BP, Wang H, Zhao YH, Liu J, Yao Y, Feng XL, Shen KL. [Molecular diagnosis of two Chinese cystic fibrosis children and literature review]. *Zhonghua Er Ke Za Zhi* 2016;54:344–8.
20 Tian X, Liu Y, Yang J, Wang H, Liu T, Xu W, Li X, Zhu Y, Xu K-F, Zhang X. p.G970D is the most frequent CFTR mutation in Chinese patients with cystic fibrosis.. *Hum Genome Var* 2016;3.
21 XH H, Liu YN, ZY L, Zhao YQ, Wang Y, Liu YQ, YM L, Liu X. A case of cystic fibrosis in children and literature review. *Int J Pediatr* 2017;44:574–9.
22 Guo ZY, Shi YY, Qian LL, Wang LB. Cystic fibrosis in infants with pseudo Bartter syndrome: a case report. *Chin J Evid Based Pediatr* 2017;12:471–3.
23 Liu K, Liu Y, Li X, Xu K-F, Tian X, Zhang X. A novel homozygous complex deletion in CFTR caused cystic fibrosis in a Chinese patient. *Mol Genet Genomics* 2017;292:1083–9.
24 CL X, RX J. Infantile cystic fibrosis complicated with pseudo Bartter syndrome: a case report and literature review. *Clin Res Pract* 2021;6:9–12.
25 Li J, Zhang Y, Wang W, Wan WL, Qiu ZQ. Cystic fibrosis in a child with pseudo Bartter syndrome: a case report and literature review. *Shandong Med J* 2017;57:48–50.
26 Zheng B, Cao L. Differences in gene mutations between Chinese and Caucasian cystic fibrosis patients. *Pediatr Pulmonol* 2017;52:E11–14.
27 Xu J, Yin Y, Zhang L, Zhang J, Yuan S, Zhang H. Four case reports of Chinese cystic fibrosis patients and literature review. *Pediatr Pulmonol* 2017;52:1020–8.
28 Sun Y, Zhong YM, Zhu M, Wang SY, Wang J, Zhang H, Zhang L, Shao H. Clinical and radiological manifestations of 5 pediatric cases with cystic fibrosis. *J Clin Pediatr* 2017;35:837–40.
29 Qiu L, Yang F, He Y, Yuan H, Zhou J. Clinical characterization and diagnosis of cystic fibrosis through exome sequencing in Chinese infants with Bartter-syndrome-like hypokalemia alkalosis. *Front Med* 2018;12:550–8.
30 XL F, Chang JB, Zeng Y, CJ X, Yang YF. A case of cystic fibrosis with pulmonary infection and hepatocirrhosis as main manifestations. *Chin J Clin Infect Dis* 2019;12:375–8.
31 Wen WF, Li W, HL L, JH X. Case report of neonatal cystic fibrosis. *Chin J Appl Clini Pediatr* 2019;34:64–5.
32 Chen LL, JZ W, Xiong H, Chen XR, Yang YG. Cystic fibrosis caused by CFTR gene variation: a case report and literature review. *J Clin Pediatr* 2019;37:457–61.
33 Weng T, YJ Q, Chen S. A case report and clinical analysis of cystic fibrosis. *Anhui Medical and Pharmaceutical Journal* 2020;24:346–8.
34 HY L, Zhu TT, Chen MG, Yang YZ, Zhang HL, XG H. Cystic fibrosis in children with pseudo Bartter syndrome: two cases report. *J Wenzhou Med Univ* 2020;50:1015–7.
35 Wang F, Yang N, Chen N, Cai XX, Shang YX. Allergic bronchopulmonary aspergillosis in childhood cystic fibrosis: a case report and literature review. *J Clin Pediatr* 2021;39:117–20.
36 MN L, Lei H, Fan J, Li M, Li L. Cystic fibrosis in 2 children with Pseudomonas aeruginosa infection. *Chin J Appl Clin Pediatr* 2021;36:300–2.
37 Yang B, Lei C, Yang D, Tan Z, Guo T, Luo H. Whole-Exome Sequencing Identified *CFTR* Variants in Two Consanguineous Families in China. *Front Genet* 2021;12:631221.
38 Wang DH, Niu C, Dai JH, Tian DY. [CFTR gene variations and phenotypes in seven children]. *Zhonghua Er Ke Za Zhi* 2021;59:689–94.
39 Wang B, Yang L. Cystic fibrosis involving multisystem: a case report and literature review. *West China Med J* 2012;27:852–4.
40 Cai Y, Chen DH, Liu WK, Zhou R. Analysis of cystic fibrosis transmembrane conductance regulator gene mutations in Chinese children with cystic fibrosis. *Chin J Appl Clin Pediatr* 2017;32:1000–3.
41 Wang Y-Q, Hao C-L, Jiang W-J, Lu Y-H, Sun H-Q, Gao C-Y, Wu M. c.753_754delAG, a novel *CFTR* mutation found in a Chinese patient with cystic fibrosis: A case report and review of the literature. *World J Clin Cases* 2019;7:2110–9.
42 Liu L-C, Shyur S-D, Chu S-H, Huang L-H, Kao Y-H, Lei W-T, Cheng C-H, Lo C-Y, Chen C-K, Fang L-C. Cystic fibrosis: experience in one institution. *J Microbiol Immunol Infect* 2014;47:358–61.
43 Wu CL, Shu SG, Zielenski J, Chiang CD, Tsui LC. Novel cystic fibrosis mutation (2215insG) in two adolescent Taiwanese siblings. *J Formos Med Assoc* 2000;99:564–7.
44 Alper OM, Shu S-G, Lee M-H, Wang B-T, Lo S-Y, Lin K-L, Chiu Y-L, Wong L-JC. Detection of novel CFTR mutations in Taiwanese cystic fibrosis patients. *J Formos Med Assoc* 2003;102:287–91.
45 Chen H-J, Lin S-P, Lee H-C, Chen C-P, Chiu N-C, Hung H-Y, Chern S-R, Chuang C-K. Cystic fibrosis with homozygous R553X mutation in a Taiwanese child. *J Hum Genet* 2005;50:674–8.
46 Wang MC, Shu SG, Chang SM, Ho WL, Chi CS. Cystic fibrosis in two Chinese infants in Taiwan. *Zhonghua Min Guo Xiao Er Ke Yi Xue Hui Za Zhi* 1993;34:314–21.
47 Chen C-H, Chang C-C, Yang B-Y, Lin PY, Wang C-S. Acute appendicitis mimicking intestinal obstruction in a patient with cystic fibrosis. *J Formos Med Assoc* 2012;111:580–3.
48 Leung GKC, Ying D, Mak CCY, Chen X-Y, Xu W, Yeung K-S, Wong W-L, Chu YWY, Mok GTK, Chau CSK, McLuskey J, Ong WPT, Leong H-Y, Chan KYK, Yang W, Chen J-H, Li AM, Sham PC, Lau Y-L, Chung BHY, Lee S-L. *CFTR* founder mutation causes protein trafficking defects in Chinese patients with cystic fibrosis. *Mol Genet Genomic Med* 2017;5:40–9.
49 Crawford J, Labrinidis A, Carey WF, Nelson PV, Harvey JS, Morris CP. A splicing mutation (1898 + 1G-->T) in the CFTR gene causing cystic fibrosis. *Hum Mutat* 1995;5:101–2.
50 Zielenski J, Markiewicz D, Lin SP, Huang FY, Yang-Feng TL, Tsui LC. Skipping of exon 12 as a consequence of a point mutation (1898 + 5G-->T) in the cystic fibrosis transmembrane conductance regulator gene found in a consanguineous Chinese family. *Clin Genet* 1995;47:125–32.
51 Wagner JA, Vassilakis A, Yee K, Li M, Hurlock G, Krouse ME, Moss RB, Wine JJ. Two novel mutations in a cystic fibrosis patient of Chinese origin. *Hum Genet* 1999;104:511–5.
52 Boon WH. Cystic fibrosis in a Chinese girl. *J Singapore Paediatr Soc* 1975;17:96–102.
53 . The clinical and functional translation of CFTR database (CFTR2), 2022. Available: https://cftr2.org/ [Accessed 29 Apr 2022].
54 Burgel PR, Munck A, Durieu I, Chiron R, Mely L, Prevotat A, Murris-Espin M, Porzio M, Abely M, Reix P, Marguet C, Macey J, Sermet-Gaudelus I, Corvol H, Bui S, Lemonnier L, Dehillotte C, Da Silva J, Paillasseur JL, Hubert D. French cystic fibrosis reference network Study Group. real-life safety and effectiveness of Lumacaftor-Ivacaftor in patients with cystic fibrosis. *Am J Respir Crit Care Med* 2020;201:188–97.
55 Bell SC, Mall MA, Gutierrez H, Macek M, Madge S, Davies JC, Burgel P-R, Tullis E, Castaños C, Castellani C, Byrnes CA, Cathcart F, Chotirmall SH, Cosgriff R, Eichler I, Fajac I, Goss CH, Drevinek P, Farrell PM, Gravelle AM, Havermans T, Mayer-Hamblett N, Kashirskaya N, Kerem E, Mathew JL, McKone EF, Naehrlich L, Nasr SZ, Oates GR, O'Neill C, Pypops U, Raraigh KS, Rowe SM, Southern KW, Sivam S, Stephenson AL, Zampoli M, Ratjen F. The future of cystic fibrosis care: a global perspective. *Lancet Respir Med* 2020;8:65–124.
56 Watson MS, Cutting GR, Desnick RJ, Driscoll DA, Klinger K, Mennuti M, Palomaki GE, Popovich BW, Pratt VM, Rohlfs EM, Strom CM, Richards CS, Witt DR, Grody WW. Cystic fibrosis population carrier screening: 2004 revision of American College of medical genetics mutation panel. *Genet Med* 2004;6:387–91.
57 Castellani C, Cuppens H, Macek M, Cassiman JJ, Kerem E, Durie P, Tullis E, Assael BM, Bombieri C, Brown A, Casals T, Claustres M, Cutting GR, Dequeker E, Dodge J, Doull I, Farrell P, Ferec C, Girodon E, Johannesson M, Kerem B, Knowles M, Munck A, Pignatti PF, Radojkovic D, Rizzotti P, Schwarz M, Stuhrmann M, Tzetis M, Zielenski J, Elborn JS. Consensus on the use and interpretation of cystic fibrosis mutation analysis in clinical practice. *J Cyst Fibros* 2008;7:179–96.
58 Martínez-Hernández A, Larrosa J, Barajas-Olmos F, García-Ortíz H, Mendoza-Caamal EC, Contreras-Cubas C, Mirzaeicheshmeh E, Lezana JL, Orozco L. Next-Generation sequencing for identifying a novel/de novo pathogenic variant in a Mexican patient with cystic fibrosis: a case report. *BMC Med Genomics* 2019;12:68.
59 Scotet V, Barton DE, Watson JBG, Audrézet M-P, McDevitt T, McQuaid S, Shortt C, De Braekeleer M, Férec C, Le Maréchal C. Comparison of the CFTR mutation spectrum in three cohorts of patients of Celtic origin from Brittany (France) and ireland. *Hum Mutat* 2003;22:105.
60 Veltman JA, Brunner HG. De novo mutations in human genetic disease. *Nat Rev Genet* 2012;13:565–75.
61 Casals T, Ramos MD, Giménez J, Larriba S, Nunes V, Estivill X. High heterogeneity for cystic fibrosis in Spanish families: 75 mutations account for 90% of chromosomes. *Hum Genet* 1997;101:365–70.
62 Bergougnoux A, Délétang K, Pommier A, Varilh J, Houriez F, Altieri JP, Koenig M, Férec C, Claustres M, Lalau G, Bienvenu T, Audrézet MP, Pagin A, Girodon E, Raynal C, Taulan-Cadars M. Functional characterization and phenotypic spectrum of three recurrent disease-causing deep intronic variants of the CFTR gene. *J Cyst Fibros* 2019;18:468–75.
63 De Boeck K, Zolin A, Cuppens H, Olesen HV, Viviani L. The relative frequency of CFTR mutation classes in European patients with cystic fibrosis. *J Cyst Fibros* 2014;13:403–9.
64 . Cystic fibrosis mutation database, 2022. Available: http://www.genet.sickkids.on.ca/cftr/StatisticsPage.html [Accessed 07 Jun 2022].
65 Amato F, Scudieri P, Musante I, Tomati V, Caci E, Comegna M, Maietta S, Manzoni F, Di Lullo AM, De Wachter E, Vanderhelst E, Terlizzi V, Braggion C, Castaldo G, Galietta LJV. Two CFTR mutations within codon 970 differently impact on the chloride channel functionality. *Hum Mutat* 2019;40:742–8.
66 Terlizzi V, Amato F, Castellani C, Ferrari B, Galietta LJV, Castaldo G, Taccetti G. Ex vivo model predicted in vivo efficacy of CFTR modulator therapy in a child with rare genotype. *Mol Genet Genomic Med* 2021;9:e1656.
67 Mattar ACV, Leone C, Rodrigues JC, Adde FV. Sweat conductivity: an accurate diagnostic test for cystic fibrosis? *J Cyst Fibros* 2014;13:528–33.
68 Sezer RG, Aydemir G, Akcan AB, Paketci C, Karaoglu A, Aydinoz S, Bozaykut A. Nanoduct sweat conductivity measurements in 2664 patients: relationship to age, arterial blood gas, serum electrolyte profiles and clinical diagnosis. *J Clin Med Res* 2013;5:34–41.