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Original Article

Multicenter Surveillance of Cystic Fibrosis in Korean Children

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ABSTRACT

Purpose: Cystic fibrosis (CF), caused by mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene, is rare among non-Caucasians. We aimed to identify the clinical features and *CFTR* mutations in Korean children.
Methods: We included 18 pediatric patients with CF diagnosed using sweat chloride test or genetic analysis for 30 years. HEK293 cells were transfected with wild-type *CFTR*, ΔF508-*CFTR*, and L441P-*CFTR* mutant plasmids for 24 hours and treated with CFTR correctors (VX809 and VX661).
Results: The median age at diagnosis was 9.2 years. Eleven patients had growth retardation, and 6 had a respiratory failure at diagnosis. Genetic analysis was used for all patients, while sweat testing was for 8 patients. At diagnosis, the median *z* scores of forced expiratory volume in one second (FEV1), FEV1/forced vital capacity, and forced expiratory flow at 25%–75% of forced vital capacity were −3.61 (−5.78, 1.78), −3.38 (−4.40, −0.60), and −4.45 (−5.78, 0.54), respectively. Two patients were treated with dornase alfa and only one with CFTR modulator. Patients were followed up for 3.7 years as a median. Four patients died at 10.6 years, with 4.2 years of post-diagnosis survival. The most common mutation was exon 16-17b deletion (19.4%). Among 11 single nucleotide variants, c.1322T>C (p.Leu441Pro, L441P) was detected in 4 patients. In the functional assay, L441P-CFTR correction was well restored by CFTR correctors compared with ΔF508.
Conclusions: CF is extremely rare in Korean children and is caused by different mutations from those commonly observed in Caucasians. Early diagnosis and treatment availability may improve outcomes. CFTR modulators may be effective for Asian patients with rare *CFTR* mutations, c.1322T>C (p.Leu441Pro).

Keywords: Cystic fibrosis; cystic fibrosis transmembrane conductance regulator; mutation; child; Korea

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INTRODUCTION

Cystic fibrosis (CF) is a rare autosomal recessive disease involving multiple organs, especially the lungs and digestive organs. It is caused by mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene in chromosome 7, which leads to alterations in a channel protein.[1,2] Over 2,100 *CFTR* mutations have been reported in the Cystic Fibrosis Mutation Database.[3] CF is most common among Caucasians, affecting approximately 1:4,000 newborns in the United States (US) and having a higher prevalence in some European countries.[4] Although the epidemiology of CF among Caucasians in Western countries is established, the reported CF incidence in Asian countries widely varies from 1:10,000 to 1:40,750; specifically, there are much fewer CF cases and a relatively higher morbidity.[5,6] This low incidence has led to decreased awareness among physicians and delayed accurate diagnosis.

In the US, the approximate CF incidence is 1:3,200, 1:10,000, 1:10,500, 1:15,000, 1:30,000 in Caucasians, Hispanics, Native Americans, African Americans, and Asian Americans, respectively.[7,8] In Asia, the CF incidence varies across countries. For example, the incidence rates are 1:2,500 in Jordan[9] and 1:5,000 in Bahrain,[10] respectively, which is similar to those in Caucasians between 1:2,000 and 1:4,000.[11] In contrast, the CF incidence in Japan is as low as 1:350,000 live births.[5] The among-country differences in the reported CF incidence could be attributed to under-reporting, under-diagnosis, lack of national registries, and mutational heterogeneity of the *CFTR* gene.

Differences in clinical manifestations between Western and Asian countries could influence CF diagnosis. A CF diagnosis is clinically confirmed when individuals have one or more distinctive phenotypic features consistent with CF and evidence of CFTR dysfunction (increased sweat chloride concentration, 2 disease-causing *CFTR* mutations, or abnormal nasal potential difference). Most patients are diagnosed with CF after symptom presentation. During the past decade, there has been a dramatic increase in the number of CF cases identified before symptom presentation due to the expansion of newborn screening (NBS) programs. For infants with positive NBS results, sweat chloride testing (SCT) is performed to clarify the CF diagnosis. Although NBS and the SCT are essential for CF diagnosis in Western countries, they are not widely applied in most Asian countries. In Korea, NBS is not performed and the SCT is only conducted for research purposes. Instead, confirming *CFTR* mutations is considered the most practical way to diagnose CF in this region. Worldwide, the most common *CFTR* gene mutation is F508del (c.1521_1523delCTT, p.Phe508del), which is found in approximately 70% of patients with CF.[12] Similarly, it is the most common mutation in Asian countries, with a few exceptions.[13]

Among Korean patients, 20 cases have been described, with the first case being diagnosed in 1988.[14-18] However, there have been no Korean studies on clinical features and *CFTR* gene mutations. This study aimed to describe the clinical features and *CFTR* mutations in Korean pediatric cases of CF. Further, we investigated whether CFTR modulators were effective for the most common single nucleotide variant (SNV) through a functional assay.

MATERIALS AND METHODS

Study population

In this pilot study, the Korean Academy of Pediatric Allergy and Respiratory Disease recruited 5 tertiary referral hospitals that accounted for 21.1% (10,924/51,787) of inpatient beds in 45 Korean tertiary hospitals, as reported by the Health Insurance Review & Assessment Service (https://www.hira.or.kr/eng/main.do). We collected the medical records of patients at the Department of Pediatric Pulmonology who had been diagnosed with CF through International Classification of Diseases 10th Revision codes related to CF (E840, E841, E848, and E849) from January 1, 2000, to December 31, 2019. Subsequently, we screened patients who met the CF diagnostic criteria based on the 2017 consensus guidelines by the Cystic Fibrosis Foundation.[4] Specifically, the criteria include patients with clinical features or a family history of CF with elevated sweat chloride concentration (≥ 60 mmol/L) or intermediate sweat chloride concentration (30–59 mmol/L) with 2 causative mutations or CFTR dysfunction identified through CFTR physiologic tests. CF is considered less likely in individuals with typical clinical features that may be consistent with CF and a sweat chloride concentration ≤ 29 mmol/L.

Data collection

A total of 18 cases were included in this study and the first diagnosis was in 1988. We collected the following demographic and clinical data: 1) basic information including sex, age at diagnosis, age at onset of symptoms, growth profiles at diagnosis, and family history; 2) respiratory tract findings including acute/persistent respiratory abnormalities, sinusitis/nasal polyps, exercise intolerance, and sputum culture as well as lung function test parameters including predicted percent values of forced vital capacity (FVC), forced expiratory volume in one second (FEV1), FEV1/FVC, and forced expiratory flow at 25%–75% of forced vital capacity (FEF25–75); 3) digestive tract findings including meconium ileus or peritonitis, ileal obstruction, rectal prolapse, hepatobiliary problems, steatorrhea/malabsorption, and malnutrition or failure to thrive; 4) other findings including hyperglycemia, glucosuria, acute pancreatitis, and delayed puberty; 5) imaging modalities including simple radiographs (chest and paranasal sinus) and chest computed tomography. We analyzed *CFTR* gene mutations and performed trio genetic analyses of the family pedigree to determine the genetic spectrum in patients with CF. Variants were categorized according to the ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) as 'Pathogenic' (variant reported as pathogenic), 'Benign/Likely benign' (variant reported as nonpathogenic or probable nonpathogenic), 'Not provided' (variant reported with missing pathogenic information), and 'Unreported' (variant unreported on the website). The sweat chloride concentration was measured by a quantitative pilocarpine iontophoresis sweat test, in accordance with the guidelines of the Clinical and Laboratory Standards Institute.[19]

Functional assay

HEK293 cells were maintained in Dulbecco's modified Eagle's medium-HG (Invitrogen, Waltham, MA, USA) supplemented with 10% fetal bovine serum (16000044; Thermo Fisher Scientific, MA, USA) and penicillin (50 IU/mL)/streptomycin (50 μg/mL) (15140122; Thermo Fisher Scientific, MA, USA). Plasmids encoding wild-type *CFTR*, ΔF508-*CFTR*, or L441P-*CFTR* mutant were transiently transfected into HEK293 cells using TransIT-X2® transfection reagent (MIR 6003; Mirus Bio, Madison, WI, USA). After 24 hours, the cells were treated with VX809 (3 or 10 μM; Vertex, Boston, MA, USA) or VX661 (3 μM; Vertex) for 24 hours. For immunoblotting, the cells were lysed using an ultrasonic homogenizer with

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lysis buffer (50 mM Tris-HCl [pH 7.4], 150 mM NaCl, 1% [v/v] Nonidet P-40, 0.25% [v/v] sodium deoxycholate, and complete protease inhibitor mixture [04693116001; Roche Applied Science, Penzberg, Germany]). After centrifugation, protein samples were separated through sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred to a nitrocellulose membrane, and immunoblotted with CFTR antibody (M3A7; Millipore, Burlington, MA, USA). Aldolase A was blotted as a loading control. Protein bands were detected through enhanced chemiluminescence (Amersham® RPN2106; GE Healthcare, Chicago, IL, USA).

Data description

Statistical analyses were performed using SPSS version 23.0 software (IBM SPSS, IBM Corp., Armonk, NY, USA). For descriptive analysis, continuous variables are presented as the median and extreme values while categorical variables are presented as either absolute values or percentages of the total.

Ethics statement

This study was approved by the Institutional Review Boards of Pusan National University Yangsan Hospital (No. 05-2020-211), Severance Hospital (No. 4-2020-0943), Asan Medical Center (No. 2020-1381), Seoul National University Hospital (No. 2008-170-1151), and Samsung Medical Center (No. 2020-10-043).

RESULTS

Clinical characteristics, laboratory findings, and treatment

The median age at diagnosis was 9.2 years (ranging from 4 months to 19 years), and 7 (38.9%) were male. Growth retardation in weight, height, and both were observed in 11 (61.1%), 7 (38.9%), and 6 (33.3%) patients, respectively. Furthermore, 6 (33.3%) patients had a respiratory failure at diagnosis. Patients were followed up for 3.7 years as a median. Four (22.2%) patients died at 10.6 years with a post-diagnosis survival period of 4.2 years (**Table 1**).

Regarding typical clinical features, 18 (100%) and 15 (83.3%) patients had respiratory and digestive symptoms, respectively. Further, 2, 1, and 1 patients showed hyperglycemia, glucosuria, and delayed puberty, respectively. Among 15 patients whose family history was investigated, 4 (26.7%)—2 siblings in each of 2 familes—had a history of CF in their siblings. Among 8 (44.4%) patients who underwent SCT, 7 (87.5%) showed increased sweat chloride concentration. Two causative *CFTR* mutations were observed in 16 (88.9%) patients (**Table 2**).

Table 1. Participant characteristics at diagnosis and follow-up (n = 18)

Clinical features	Value
Age (yr) at diagnosis	9.2 (0.4, 19.2)
Male	7 (38.9)
Growth retardation at diagnosis	
Weight < 3 percentile	11 (61.1)
Height < 3 percentile	7 (38.9)
Respiratory failure at diagnosis	6 (33.3)
Follow-up period (yr)	3.7 (0.4, 14.3)
Deaths*	4 (22.2)
Age (yr) at death	10.6 (5.8, 16.8)
Survival period (yr)	4.2 (0.4, 6.9)

Data are presented as number (%) and median (minimum, maximum).
*The causes of death were pneumonia (n = 1), septic shock (n = 1), rejection after lung transplantation (n = 1), and liver failure/cerebral hemorrhage (n = 1).

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Table 2. Clinical and laboratory findings at diagnosis (n = 18)

Clinical and laboratory findings at diagnosis	Value
Findings applied for the diagnostic criteria	
Typical clinical features	
Respiratory tract	18 (100.0)
Digestive tract	15 (83.3)
Others*	3 (16.7)
A history of CF in a sibling (n = 15)	4 (26.7)
Sweat chloride concentration ≥ 60 mmol/L (n = 8)†	7 (87.5)
Two mutations known to cause CF on separate alleles‡	16 (88.9)
Respiratory findings at diagnosis	
Chest CT	
Bronchiectasis	14 (77.8)
Cyst formation	1 (5.6)
Spirometry (n = 10)	
FEV1 *z* score	−3.61 (−5.78, 1.78)
FEV1/FVC *z* score	−3.38 (−4.40, −0.60)
FEF25–75 *z* score	−4.45 (−5.78, 0.54)
Sputum culture (n = 15)	
Pseudomonas aeruginosa	10 (66.7)
Staphylococcus aureus	6 (40.0)
Others§	6 (40.0)

Data are presented as number (%) and median (minimum, maximum).
CT, computed tomography; FEV1, forced expiratory volume in one second; FVC, forced vital capacity; FEF25–75, forced expiratory flow at 25%–75% of forced vital capacity.
*Other features included hyperglycemia (n = 2), glucosuria (n = 1), and delayed puberty (n = 1).
†Sweat chloride test was performed in 8 patients but failed in 1.
‡Using mutation classifications identified in the ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/).
§Other bacterial organisms were *Haemophilus influenzae* (n = 2), *Stenotrophomonas maltophilia* (n = 2), *Streptococcus pneumoniae* (n = 1), and *Achromobacter xylosoxidans* (n = 1).

Genetic data regarding trio family pedigrees were collected from 11 patients in 10 families (**Supplementary Fig. S1**). All patients showed respiratory symptoms at diagnosis while 14 (77.8%) patients showed bronchiectasis on chest computed tomography. The patients' spirometry showed pulmonary obstructive patterns. Specifically, the median *z* scores of FEV1, FEV1/FVC, and FEF25–75 were −3.61 (−5.78, 1.78), −3.38 (−4.40, −0.60), and −4.45 (−5.78, 0.54), respectively. *Pseudomonas aeruginosa* was the most frequently isolated pathogen (n = 10), followed by *Staphylococcus aureus* (n = 6), *Haemophilus influenzae* (n = 2), *Stenotrophomonas maltophilia* (n = 2), *Streptococcus pneumoniae* (n = 1), and *Achromobacter xylosoxidans* (n = 1) (**Table 2**).

CF treatments mainly comprised airway clearance and antibiotic therapy. Primary airway clearance therapy, including chest physiotherapy and inhaled *N*-acetylcysteine; hypertonic saline nebulization; and specific inhalation solution—dornase alfa—were applied in 15, 8, and 2 patients, respectively. Azithromycin (n = 10) and inhaled tobramycin (n = 7) were administered for patients with *P. aeruginosa*. CFTR modulator therapy and lung transplantation were performed for 1 and 3 patients, respectively (**Supplementary Table S1**).

CFTR gene mutations

Table 3 summarizes the *CFTR* gene variants in each patient. Ten patients had been described in previous studies.[17-20] Two sets of siblings in each family had the same biological parents. Six patients carried 2 causative 'Pathogenic' variants while twelve patients carried more than one 'Pathogenic' variant. Among patients without 'Pathogenic' variants, 4 met the SCT diagnostic criteria (≥ 60 mmol/L). **Table 4** indicates the frequencies of *CFTR* gene mutations. There were 10, 1, 2, and 5 'Pathogenic', 'Benign/Likely benign', 'Not provided', and 'Unreported', respectively. Five mutation types were identified: SNV, deletion, duplication, insertion, and

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Table 3. Results of *CFTR* gene testing and the sweat chloride test in 18 Korean patients with CF

Patient No.	Age at diagnosis	Sex	First variant		Second variant		Sweat test (mmol/L)	Reference
			cDNA name	Protein name	cDNA name	Protein name		
1*	14 yr 11 mon	Female	**Exon 16-17b deletion**	-	**Exon 16-17b deletion**	-	ND	17
2	9 yr 2 mon	Male	**Exon 16-17b deletion**	-	**Exon 16-17b deletion**	-	103.7	
3*	6 yr 3 mon	Male	**Exon 16-17b deletion**	-	Exon 14a deletion	-	ND	17
4a*	14 yr 1 mon	Male	**Exon 16-17b deletion**	-	c.3871C>T	p.Gln1291Ter	ND	17
5a*	19 yr 3 mon	Male	**Exon 16-17b deletion**	-	c.3871C>T	p.Gln1291Ter	ND	17
6*	9 yr 2 mon	Male	**Exon 16-17b deletion**	-	**c.3196C>T**	p.Arg1066Cys	ND	17
7	5 yr 8 mon	Male	**Exon 16-17b deletion**	-	**c.1657C>T**	p.Arg553Ter	ND	
8	13 yr 8 mon	Female	**Exon 16-17b deletion**	-	c.2052del	p.Lys684fs	ND	
9b	7 yr 11 mon	Female	c.1322T>C	p.Leu441Pro	c.223C>T	p.Arg75Ter	123	
10b	9 yr 4 mon	Female	c.1322T>C	p.Leu441Pro	c.223C>T	p.Arg75Ter	Fail	
11	7 mon	Female	c.1322T>C	p.Leu441Pro	c.273+2T>A	-	ND	
12*	5 yr	Female	c.1322T>C	p.Leu441Pro	-	-	88.7	18
13	9 yr 8 mon	Female	**c.2977G>T**	p.Asp993Tyr	**c.658C>T**	p.Gln220Ter	71.1	
14	13 yr 3 mon	Female	**c.2977G>T**	p.Asp993Tyr	**c.263T>G**	p.Leu88Ter	ND	
15*	5 yr 11 mon	Female	c.2562T>G	p.Thr854 =	c.2562T>G	p.Thr854 =	97.5	19
16*	4 mon	Male	**c.263T>G**	p.Leu88Ter	c.2089_2090insA	p.Arg697LysfsX33	ND	20
17*	10 mon	Female	**c.3908dupA**	p.Asn1303fs	**c.1766+2T>C**	-	102	17
18*	13 yr 9 mon	Female	c.3871C>T	p.Gln1291Ter	IVS8-5T	p.Met470Val	108.1	19

Bold indicates the 'Pathogenic' variant in the ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/).
CFTR, cystic fibrosis transmembrane conductance regulator; CF, cystic fibrosis; ND, not done.
*Previously reported cases.
[a]Patient 3 and 4 are siblings that share the same biological parents.
[b]Patient 7 and 8 are siblings that share the same biological parents.

Table 4. Frequencies of *CFTR* gene mutations and variants present in Korean patients with CF

Mutation type	cDNA name	Protein name	Chr 7 position*	dbSNP ID	Mutations, n (%)	Clinical significance in the ClinVar†
SNV	c.1322T>C	p.Leu441Pro	117548753	rs397508188	4 (11.1)	Not provided
	c.3871C>T	p.Gln1291Ter	117642591	rs397508620	3 (8.3)	Not provided
	c.223C>T	p.Arg75Ter	117509092	rs121908749	2 (5.6)	Pathogenic
	c.2977G>T	p.Asp993Tyr	117606742	rs397508468	2 (5.6)	Pathogenic
	c.263T>G	p.Leu88Ter	117509132	rs397508412	2 (5.6)	Pathogenic
	c.1657C>T	p.Arg553Ter	117587811	rs74597325	1 (2.8)	Pathogenic
	c.3196C>T	p.Arg1066Cys	117611637	rs78194216	1 (2.8)	Pathogenic
	c.658C>T	p.Gln220Ter	117535326	rs397508778	1 (2.8)	Pathogenic
	c.1766+2T>C	-	117590441	rs1554389062	1 (2.8)	Pathogenic/Likely pathogenic
	c.2562T>G	p.Thr854 =	117595001	rs1042077	2 (5.6)	Benign/Likely benign
	c.273+2T>A	-	-	-	1 (2.8)	Unreported
Deletion	Exon 16-17b deletion	-	-	-	10 (27.7)	Pathogenic
	c.2052del	p.Lys684fs	117592213	rs121908746	1 (2.8)	Pathogenic
	Exon 14a deletion	-	-	-	1 (2.8)	Unreported
Duplication	c.3908dupA	p.Asn1303fs	117652876	rs397508637	1 (2.8)	Pathogenic
Insertion	c.2089_2090insA	p.Arg697LysfsX33	-	-	1 (2.8)	Unreported
Intron variant	IVS8-5T	p.Met470Val	-	-	1 (2.8)	Unreported

CFTR, cystic fibrosis transmembrane conductance regulator; CF, cystic fibrosis; SNV, single nucleotide variant; Chr, chromosome.
*Position of variants in GRCh38-hg38.
†Reporting clinical significance identified in the ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/).

intron variant. Exon 16-17b deletion (n = 10, 27.7%) was the most common mutation. The most common SNV was c.1322T>C (n = 4, 11.1%), followed by c.3871C>T (n = 3).

CFTR modulator

Figure shows the functional assay. Most wild-type *CFTR* proteins were detected in the fully glycosylated mature form (band C). Almost all L441P mutant proteins (*e.g.*, ΔF508-CFTR) appeared in the core-glycosylated form around 150 kDa (band B). Compared with ΔF508L441, P-CFTR correction was well restored by the CFTR corrector (VX809 and VX661). VX809 and

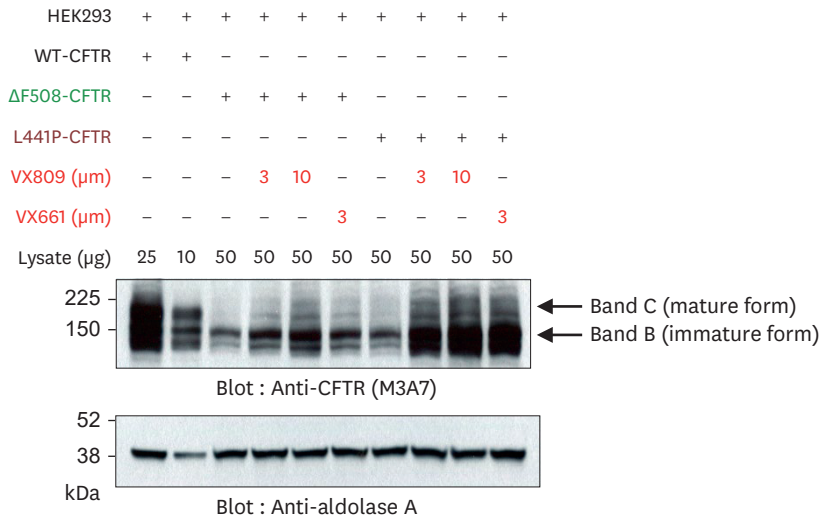

Figure. Rescue of L441P using VX809. Aldolase A was blotted as a loading control. Most wild-type *CFTR* proteins were detected in the fully glycosylated mature form (band C), whereas virtually all L441P mutant proteins appeared in the core-glycosylated form of around 150 kDa (band B). When treated with VX809, L441P showed better restoration of the folding defect than ΔF508.
CFTR, cystic fibrosis transmembrane conductance regulator.

VX661 increased the abundance of mature and immature L441P-CFTR forms. After treatment with VX809, L441P revealed better folding defect restoration, even compared with ΔF508.

DISCUSSION

This study described the current situation of Korean children with CF. In Korea, CF was extremely rare with exclusive *CFTR* mutations, which limited diagnosis or treatment. Additionally, CF was diagnosed at an old age in Korea compared to Western countries, leading to poor clinical outcomes with respect to respiratory mortality and morbidity.

Most Korean physicians do not encounter patients with CF. They did not suspect CF even in patients with chronic lung diseases, including recurrent pneumonia or bronchiectasis. The patients with exon 16-17b deletion of CFTR gene had been reported to experience postnatal CF-related symptoms; however, they were diagnosed with CF between the age of 8 and 19 years.[17] Only a small number of CF cases have been reported in Korea.[14,18,20-24] We also confirmed that Korean CF patients were extremely rare in the present study, with some patients previously reported.[16,17,25] Accordingly, the rarity caused the unavailability of a CF-specific diagnostic tool, SCT. However, we have noticed that half of the patients were diagnosed through CFTR sequencing since 2015, who could not have been diagnosed only using the traditional gene panel test before. Currently, more CF cases may also be detected among adult patients with end-stage chronic lung diseases using CFTR sequencing, who could not have been diagnosed in the past. Taken together, more recognition of CF with diagnostic effort, including the availability of SCT, could allow the identification of more Korean CF cases.

NBS for CF has made early diagnosis possible at the age of 0.5 months in Europe[26] and 3 months in America,[27] and improved the prognosis in Western countries. The lack of NBS in

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Korea may lead to delayed CF diagnosis and under-recognition by physicians. Notably, all our patients had chronic respiratory symptoms; moreover, more than half of the children had bronchiectasis, abnormal pulmonary function, and *P. aeruginosa* in their sputum at the time of diagnosis. In contrast, only 39.1% had pancreatic insufficiency, which is lower than the rate in Caucasians (approximately 85%).[28] These distinctive clinical characteristics could be attributed to differences in ethnicity or mutation sites. Further, the performance of NBS could influence the disparity of clinical features and diagnosis timing.

There are differences in *CFTR* mutation between Caucasians and Asians (https://cftr2.org/). We did not identify cases with the F508del mutation. Further, there were differences in the panels developed for population screening.[29] For example, among the 23 most common mutations, which comprise approximately 84% of CF-causing mutations, we only identified 2 mutations (c.1657C>T, p.Arg553Ter; c.2052delA, p.Lys684fs). Exon 16-17b deletion has been identified as the most common mutation in Korean CF cases. The second most common mutation was c.1322T>C (p.Leu441Pro) variant, which was found in Japan and Korea,[20,30] but has not be described in the ClinVar. Our top 3 common mutations (exon 16-17b deletion; c.1322T>C, p.Leu441Pro; c.3871C>T, p.Gln1291Ter) were mostly restricted to Asian patients.[17,30,31] We observed sufficient L441P-CFTR L441P-CFTR correction by VX809 and VX661.[17] Our findings indicate that CFTR modulators can functionally improve CFTR channels in patients with the p.Leu441Pro mutation. Future studies should assess their feasibility for unestablished mutations.

Successful CF treatment requires early diagnosis and individualized treatment. Under-diagnosis of CF in Korea could be attributed to its rarity; the absence of a screening system; and lack of diagnostic and treatment resources, which leads to misdiagnosis and CF progression. Current treatment strategies for CF in Korea mainly focus on nutritional support and preventing acute respiratory exacerbations. In our study, 4 patients had died. The range of death age was 5.8–16.8 years, which considerably differs from the median predicted survival age of 46.2 years for individuals born from 2015 to 2019 in the US.[27] The life expectancy of patients with CF has been increased by CFTR modulators, which act by boosting production, intracellular processing, and the function of the defective CFTR protein. These drugs have advanced CF management since they target the production/function of the mutant CFTR protein rather than its downstream consequences.[32] Our findings suggest that c.1322T>C (p.Leu441Pro) mutation could be a gating variant, with CFTR correctors improving the prognosis of carrier patients.

As the first large-scale survey in Korea, this study summarized CF cases enrolled from the top 5 tertiary referral hospitals, showed the clinical characteristics of Korean CF patients and highlighted the significant differences in the spectrum of *CFTR* mutations in the Korean population. These 5 hospitals are considered representative institutions in Korea—treating most patients with genetic and severe chronic disorders and accounting for about 20% of the number of inpatient beds among 45 tertiary hospitals in Korea. That means our study can be representative of CF in Korean pediatric patients. Moreover, we provided basic data for expanding the indications of CFTR modulators through the functional assay, while most previous studies on CF cases in Asian countries only reported trio results. We also acknowledge that there are limited data regarding the CF prevalence in Korea given the small number of patients and various ascertainment biases. Since patients with respiratory symptoms might be mainly selectively even in tertiary referral hospitals, patients without respiratory problems might be underdiagnosed. In addition, in patients with exon 16-17b

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deletion, limited genetic testing in the past may have failed to identify patients with large deletions despite their typical phenotypes. Lastly, another limitation of this study is that we obtained data in a retrospective way depending on the medical records. Nevertheless, this study has great significance since it is first large-scale survey of CF in Korea.

In conclusion, our findings contribute to the elucidation of CF cases in Korea with respect to the clinical features, mutational spectrum of *CFTR* gene, and treatment strategy. Our findings indicated that CF is extremely rare in Korean children and is caused by different mutations from those commonly observed in Caucasians. Early diagnosis and SCT availability may improve clinical outcomes in patients with CF. Furthermore, CFTR modulators can be effective for Asian patients with rare and unreported *CFTR* mutations. Our findings can help Korean physicians understand the clinical features and *CFTR* mutations in Korean patients with CF, which may facilitate early diagnosis and advance CF treatments.

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SUPPLEMENTARY MATERIALS

Supplementary Table S1

Treatments for patients with cystic fibrosis at diagnosis

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Supplementary Fig. S1

Genetic data of trio family pedigrees. The patient numbering is consistent with them in **Table 3**.

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