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ARTICLE

Clinical and genetic features of cystic fibrosis in Japan

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Cystic fibrosis (CF) is an autosomal recessive disease caused by pathogenic variants in *CF transmembrane conductance regulator* (*CFTR*). While CF is the most common hereditary disease in Caucasians, it is rare in East Asia. In the present study, we have examined clinical features and the spectrum of *CFTR* variants of CF patients in Japan. Clinical data of 132 CF patients were obtained from the national epidemiological survey since 1994 and CF registry. From 2007 to 2022, 46 patients with definite CF were analyzed for *CFTR* variants. All exons, their boundaries, and part of promoter region of *CFTR* were sequenced and the presence of large deletion and duplications were examined by multiplex ligation-dependent probe amplification. CF patients in Japan were found to have chronic sinopulmonary disease (85.6%), exocrine pancreatic insufficiency (66.7%), meconium ileus (35.6%), electrolyte imbalance (21.2%), CF-associated liver disease (14.4%), and CF-related diabetes (6.1%). The median survival age was 25.0 years. The mean BMI percentile was 30.3%ile in definite CF patients aged < 18 years whose *CFTR* genotypes were known. In 70 CF alleles of East Asia/Japan origin, CFTR-dele16-17a-17b was detected in 24 alleles, the other variants were novel or very rare, and no pathogenic variants were detected in 8 alleles. In 22 CF alleles of Europe origin, F508del was detected in 11 alleles. In summary, clinical phenotype of Japanese CF patients is similar to European patients, but the prognosis is worse. The spectrum of *CFTR* variants in Japanese CF alleles is entirely different from that in European CF alleles.

Journal of Human Genetics (2023) 68:671–680; https://doi.org/10.1038/s10038-023-01160-2

INTRODUCTION

Cystic fibrosis (CF) is an autosomal recessive disease caused by variants in *CF transmembrane conductance regulator* (*CFTR*) gene. CFTR is expressed in the apical membrane of various epithelia and functions as a cyclic AMP-regulated anion channel. Loss of function due to severe pathogenic variants in both alleles causes dehydrated, thick, and viscous luminal fluid in respiratory and gastrointestinal tract, pancreatic duct, and vas deferens. CF typically involves chronic infection and progressive obstruction of the respiratory tract, pancreatic exocrine insufficiency (PI), and male infertility. CFTR mediates Cl^- absorption by the sweat duct and elevated sweat Cl^- is the gold standard for CF diagnosis.

While CF is one of the most common hereditary diseases in Europeans with an estimated incidence of one in 2500–3500 newborns [1, 2], CF is very rare in East Asia. The incidence of CF in Japan is approximately 1 in 590,000 live births [3]. In China, 78 patients were reported during 2015–2019 (ref. [4]). Sporadic cases and case series in institutions were reported from Korea and Taiwan [5, 6]. Meanwhile, it has been reported that the presence of *CFTR* variants increases the risk of chronic pancreatitis, bronchiectasis, and male infertility in Japan, Korea, and China [7–11].

Clinical phenotype and prognosis of CF in East Asia are not well understood and only a few studies are found in the literature. Asians with CF in UK had a worse clinical course (lung function and nutritional status) compared to clinic-matched non-Asian patients homozygous for F508del [12]. Impact of Asian ethnicity on the diagnosis of CF was recently examined by using the data accumulated in CFTR2 (Clinical and Functional Translation of CFTR; https://cftr2.org/) and the UK CF database [13]. Asians with CF did not have a worse clinical phenotype (no difference in lung function), while pancreatic exocrine sufficiency (PS) was more common and more patients had sweat chloride values lower than 60 mmol/L.

More than 2000 different variants have been reported to the Cystic Fibrosis Mutation Database (CFMD, www.genet.sickkids.on.ca/cftr). CF-causing variants are generally classified to 5 major classes: defective protein production (I), defective protein processing (II), defective regulation (III), defective conduction (IV), and splicing variants (V) [14]. However, the functional consequences and disease liability of most of the rare *CFTR* variants are unknown [15]. Diversity in the spectrum of *CFTR* variants among ethnic groups and regions has long been known. Common CF-causing variants in Europeans, such as F508del, have rarely been identified in CF patients in East

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Received: 10 February 2023 Revised: 28 April 2023 Accepted: 7 May 2023
Published online: 22 May 2023

Asia [4, 5, 16–18]. Most of the *CFTR* variants found in CF alleles inherited from Japanese ancestry were of rare types [16, 17].

In the present study, we have analyzed the clinical features and *CFTR* variants of CF patients in Japan. This is the first comprehensive and nationwide study of clinical and genetic features of CF in an East Asian country.

MATERIALS AND METHODS

This study was approved by the Ethical Committee of Nagoya University Graduate School of Medicine (No. 2008-0650, No. 2012-0310). Written informed consent was obtained from each subject or guardian prior to the study.

Subjects

Clinical data of 140 patients with CF were obtained by the national epidemiological survey conducted at 1994, 1999, 2004, 2009, and 2014 by the Research Committee of Intractable Pancreatic Diseases provided by the Ministry of Health, Labour and Welfare of Japan and from the CF registry Japan established in 2012. Eight patients who were not born in Japan are excluded. The other 132 patients (65 males and 67 females) are analyzed in the present study (Table 1). The last patient was registered in December 2022. Diagnosis of CF was based on the criteria by the Research Committee of Intractable Pancreatic Diseases, Japan: diagnosed as definite CF when 1) characteristic respiratory symptoms were present with positive sweat test ($[Cl^-] \geq 60$ mmol/L), 2) 2 or more of PI, meconium ileus, and family history of CF were present with positive sweat test, or 3) 1 or more clinical symptoms were present and 2 pathogenic *CFTR* variants were detected; diagnosed as probable CF when 1) PI or meconium ileus was present with positive sweat test, 2) characteristic respiratory symptoms were present with borderline sweat test (40–59 mmol/L, 30–59 mmol/L under 6 months of age), 3) 2 or more of PI, meconium ileus, and family history of CF were present with borderline sweat test, or 4) 1 or more clinical symptoms were present and 1 pathogenic *CFTR* variant was detected.

Table 1. Summary of CF patients in Japan since 1994

		All patients		Male	Female
		132	(%)	65	67
Diagnosis	Definite	116	(87.9)	56	60
	Probable	16	(12.1)	9	7
Sweat [Cl^-]	Positive	103	(78.0)	53	50
	Borderline	6	(4.5)	2	4
	Negative	4	(3.0)	1	3
	Not examined	19	(14.4)	9	10
Pancreatic exocrine function	PI	88	(66.7)	43	45
	PS	39	(29.5)	19	20
	Not examined	5	(3.8)	3	2
Chronic sinopulmonary disease		113	(85.6)	57	56
	Sinusitis	52	(39.4)	25	27
	Pulmonary disase	112	(84.8)	56	56
Meconium ileus		47	(35.6)	21	26
Family history		29	(22.0)	16	13
Electrolyte imbalance		28	(21.2)	18	10
CF-associated liver disease (CFLD)		19	(14.4)	14	5
CF-related diabetes		8	(6.1)	5	3
CF-causing *CFTR* variants	2	46	(34.8)	20	26
	1	14	(10.6)	5	9
	0	25	(18.9)	16	9
	Not examined/ unknown	47	(35.6)	24	23

Data are presented as numbers of patients (with percentage in parentheses)

Sweat test was performed by the standard method (pilocarpine iontophoresis) in most of the recent CF cases (60 patients). The standard method is currently available in only one hospital in Japan; finger sweat chloride test [19] was performed in 2 patients whereas the analytical methods for the other 51 patients are unknown.

Clinical and phenotype data

Pulmonary function was evaluated by percent predicted forced expiratory volume in 1 s (%FEV_1). PI was diagnosed by low levels of fecal pancreatic elastase (< 200 µg/g), fecal chymotrypsin, or plasma trypsin, or the presence of persistent steatorrhea. Fecal pancreatic elastase was measured by ELISA (Fecal Pancreatic Elastase ELISA, Bioserv Diagnostics, Rosrock, Germany or Pancreatic Elastase 1, Schebo, Giessen, Germany). CF-associated liver disease (CFLD) was diagnosed according to the criteria proposed by Debray et al. [20]. Nutritional status was evaluated by body mass index (BMI) for individuals aged ≥ 18 years and BMI percentile (%ile) for individuals aged < 18 years.

%FEV_1 was calculated according to the formulas for Japanese children and adults provided by the Japanese Society of Pediatric Pulmonology and the Japanese Respiratory Society. BMI %ile was calculated according to the reference data of Japanese children provided by the Japanese Society for Pediatric Endocrinology. We have not performed ethnicity adjustment, because the reference values for spirometry and growth are available only for limited countries [21, 22].

Analysis of the *CFTR* variant

From 2007 to 2022, all 27 exons and their boundaries (100–300 bp including TG repeats and poly T in intron 8) and promoter region (up to 1028 bp upstream of the translation initiation codon of exon 1) of the *CFTR* gene were sequenced in the majority of cases [17]. The presence of large deletion and duplication were examined by multiplex ligation-dependent probe amplification (MLPA) using the SALSA P091-C1 CFTR MLPA kit (MRC Holland, Amsterdam, The Netherlands).

Data analysis

Statistical analyses were carried out using X-squared test with Yates' correction, Fisher's exact test, or Student's *t*-test with significance defined as $p < 0.05$. Survival rates were calculated by the Kaplan–Meier method. Differences between survival curves were computed with the log-rank test. Data are presented as means ± SD unless otherwise indicated.

RESULTS

Phenotype and prognosis of CF in Japan

Table 1 summarizes the clinical data of 132 CF patients in Japan accumulated from 1994 to 2022. According to the diagnostic criteria, 116 patients (87.9%) were classified as having definite CF and 16 patients (12.1%) were classified as having probable CF. In CF patients, chronic sinopulmonary disease was observed in 85.6% patients, PI observed in 66.7% patients, meconium ileus observed in 35.6% patients, electrolyte imbalance in 21.2% patients, CFLD in 14.4% patients, and CF-related diabetes was found in 6.1% patients. Whereas most patients had sinus involvement with pulmonary disease, there was one mild case (one male patient) that had chronic sinusitis but not pulmonary disease. The values of sweat Cl^- were 103 ± 34 mmol/L ($n = 109$). CF-associated liver disease (CFLD) was more common in male patients than in female patients ($p < 0.05$, Table 1). Similarly, in a recent French study of CF [23], males were also reported to be at risk of CFLD. There were no significant gender differences for other clinical features.

The age of onset was 0.0 ~ 19.0 years (median: 0.3; interquartile range (IQR): 0.0–3.2; $n = 123$). The age of diagnosis was 0.0 ~ 47.6 years (median: 2.5; IQR: 0.5–10.6; $n = 123$).

The median survival age at the end of December 2012 was 18.8 years (95% confidence interval (CI): 16.6–21.1; 76 patients). In contrast, the median survival age at the end of December 2022 was 25.2 years (95% CI: 18.7–31.7). While, there was no significant difference between male (25.2 years; 95% CI: 20.1–30.3) and female (21.1 years; 95% CI: 10.7–31.5) patients, the overall survival time was significantly ($p < 0.05$) extended during the 2012–2022 period. Among 57 patients whose cause of death is known, 54 patients died of respiratory failure, 2 patients died of liver failure, and 1 patient died of variceal rupture.

CFTR variants and haplotype of definite CF patients in Japan since 2007

Table 2 shows the list of 46 cases of definite CF in order of patient registration since 2007. All exons, their boundaries, and part of the promoter region of the *CFTR* gene were sequenced and MLPA was performed. Detected *CFTR* variants are shown as legacy names along with the haplotype of TG repeats and poly T in intron 8, ethnicity or country of origin of their parents. The following clinical data are shown: swear [Cl^-], PS or PI (fecal pancreatic elastase), presence or absence of chronic pulmonary disease, %FEV_1, presence or absence of pseudomonas aeruginosa infection, meconium ileus, CFLD, and lung/liver transplant.

The haplotype spectrum of TG repeats and poly T was markedly different between the alleles of East Asia/Japan origin (70 alleles) and those of European origin (22 alleles), as we have previously reported [7]. East Asian/Japanese-type alleles had (TG)12-7 T (47 alleles) or (TG)11-7 T (20 alleles) haplotype. Twenty-one of 47 alleles with (TG)12-7 T haplotype had deletion of exons 18, 19, and 20 (CFTR-dele16-17a-17b in legacy name) (Table 3). European-type alleles had (TG)12-5 T (1 allele), (TG)11-9 T (1 allele), (TG)11-7 T (7 alleles), (TG)10-9 T (12 alleles), or (TG)10-7 T (1 allele) haplotype. Eleven of 12 alleles with (TG)10-9 T haplotype had c.1521_1523delCTT (F508del) variant (Table 3).

While pathogenic *CFTR* variants were detected in all of the European-type alleles, no pathogenic variants were detected in 8 (11%) of the East Asian/Japanese-type alleles. In some of those cases, we have tried to analyze CFTR transcripts from nasal swab [17]. In patient No. 4 where the E217G variant was detected in one allele, the amount of CFTR transcripts were reduced to 10-30% of healthy controls (data not shown). In the case of No. 9 patient who had CFTR-dele16-17a-17b in one allele, a splicing defect: deletion/skipping of exon 1 was detected in the other allele [17].

Spectrum of East Asian/Japanese-types of *CFTR* variants

Table 3 is a list of pathogenic *CFTR* variants found in 46 cases of definite CF since 2007 in descending order of allele frequency. The list includes 4 types of large deletions. Pathological significance of each variant (CF-causing class I ~ V [14], pathogenic, etc.) is described with references where available (CFTR2, CFMD, ClinVar, VarSome, or literatures).

Across 27 East Asian/Japanese-type variants, CFTR-dele16-17a-17b was most frequently detected and was present in 24 alleles. Twenty-one East Asian/Japanese-type variants were detected in one patient or one pair of siblings and 10 types were novel variants that were identified in our laboratory. Currently, 17 East Asian/Japanese variants can be classified as CF-causing, 5 variants as pathogenic, 2 variants as likely pathogenic, 1 variant as likely pathogenic/uncertain significance, 1 variant as uncertain significance, and 1 variant as likely benign.

Four types of large deletion were detected in 28 alleles. Their breakpoints were identified in our laboratory. "CFTR-dele16-17a-17b" is the deletion of ~7 kb [17]. "CFTRdele promoter" is the deletion of ~138 kb which includes exon 1, promoter region of the *CFTR* gene and all of the *ASZ1* gene [24]. "CFTRdele 2,3" and "CFTRdele23 (CFTRdele 20 in legacy name)" are deletions of ~18 kb and ~17 kb, respectively.

All of 11 European-type variants are CF-causing (Table 3) with F508del the most frequent and detected in 11 alleles.

Genotype-phenotype relationship and impact of ethnicity

In a population of 46 patients with definite CF (Table 2), 32 patients were PI and 11 patients were PS. Figure 1A shows values of sweat Cl^-, fecal pancreatic elastase, %FEV_1, SD scores of height and weight, BMI %ile, serum levels of albumin and total cholesterol, and blood hemoglobin of definite CF patients aged < 18 years ($n = 30$). There were no significant gender differences ($p > 0.05$).

CFTR-dele16-17a-17b, the most frequent East Asian/Japanese-type variant was detected in 21 patients. There were 3 patients with CFTR-dele16-17a-17b-homozygosity and 7 patients with compound heterozygote of CFTR-dele16-17a-17b and F508del or Class I (function is completely lost) variants. All 10 patients were PI and 5 patients had meconium ileus. This suggests that CFTR-dele16-17a-17b is a severe variant which is causative of classic CF. The incidence of meconium ileus, CFLD, PI, and electrolyte imbalance were not significantly different when comparing the patients who had at least one CFTR-dele16-17a-17b (dele16-17a-17b +) with those who did not have CFTR-dele16-17a-17b (dele16-17a-17b –) (Table 4). Similarly, values of sweat Cl^-, fecal pancreatic elastase, %FEV_1, and BMI %ile were not significantly different when compared in patients aged < 18 years with or without CFTR-dele16-17a-17b (Fig. 1B).

To evaluate the impact of ethnicity, phenotypes were compared between 34 patients with 2 East Asian/Japanese-type alleles (Japanese-Japanese) and 10 patients with 2 European-type alleles (European-European) (Table 4). While more Japanese-Japanese patients had meconium ileus (38%) or PS (31%) compared to European-European patients, 10% and 11% respectively, the data were not significantly different. We compared values of sweat Cl^-, fecal pancreatic elastase, %FEV_1, and BMI %ile between Japanese-Japanese and European-European patients aged < 18 years (Fig. 1C). While all 6 European-European patients showed very low levels of fecal pancreatic elastase, 4 of 20 Japanese-Japanese patients were PS (≥ 200 μg/g). When clinical data were compared between PI ($n = 26$) and PS ($n = 4$) patients aged < 18 years whose CFTR genotypes were known (Fig. 1D), the levels of serum albumin and total cholesterol, and blood hemoglobin in PI patients were significantly ($p < 0.05$) lower than those in PS patients.

We noted that BMI %ile of most Japanese-Japanese patients (81%) was below 50%ile, which suggests that poor nutritional condition is common in Japanese-Japanese CF patients.

DISCUSSION

In the present study, we have analyzed the clinical features and *CFTR* variants of CF patients in Japan. This is the first comprehensive and nationwide study on clinical and genetic features of CF in an East Asian country. The CF registry in Japan was established in 2012 to facilitate information exchange between clinicians, medical staffs, CF patients and their families. In the last 8 years, 2–9 patients have been registered each year.

The organs affected and severity of symptoms of CF differ greatly because of variable levels of CFTR dysfunction [25–27]. While classical CF is characterized by multiple-organ manifestations (bronchiectasis, sinus polyps, and PI), some patients have only atypical or isolated manifestations such as pancreatitis, respiratory symptoms without bronchiectasis, and male infertility. In the United States, CF is diagnosed when an individual has a characteristic clinical presentation and evidence of CFTR dysfunction (elevated sweat Cl^- or 2 CF-causing CFTR variants) [25]. There are 2 categories in Japanese diagnostic criteria: definite and probable CF. The Japanese criteria of "definite CF" are compatible with CF criteria in the United States. "Probable CF" may include CFTR-related disorders [28].

Table 2. Genetic and clinical features of definite CF patients since 2007

Patient	Gender	Age at onset	Age at diagnosis	Allele-1			Allele-2			Sweat Cl^-	PS/PI (Fecal elastase)	Chronic sinopulmonary disease	% FEV_1	Pseudomonas aeruginosa infection	Meconium ileus	Electrolyte imbalance	CFLD	Transplant
				Ethnicity/ Country of origin	*CFTR* variant	(TG) mTn	Ethnicity/ Country of origin	*CFTR* variant	(TG) mTn									
1	Male	10.3	32.5	Japanese	CFTR-dele 16-17a-17b	TG12T7	Japanese	T1086I	TG12T7	88		+		+	−	−	−	−
2	Male	0.0	0.4	Japanese	R75X	TG12T7	Japanese	CFTR-dele 16-17a-17b	TG12T7	96	PI (19)	+	91.2	+	+	+	−	Lung
3	Male	0.0	0.0	Japanese	L441P	TG11T7	Japanese	L441P	TG11T7	80	PI	+	22.8	+	+	−	+	Liver
4	Male	0.0	15.8	Japanese	ND	TG12T7	Japanese	E217G	TG12T7	70	PS (600)	+	20.5	+	−	−	−	−
5	Female	0.0	6.5	Japanese	ND	TG11T7	Japanese	L441P	TG11T7	114	PS (2,531)	+	104.4	+	+	+	−	Lung
6	Female	0.0	0.3	Japanese	G85R	TG12T7	Japanese	CFTR-dele 16-17a-17b	TG12T7	108	PI (0)	+	72.5	−	+	+	−	−
7	Female	0.5	1.5	Peruvian	G542X	TG10T9	Peruvian	1609delCA	TG11T7	150	PI (1)	+	74.9	−	−	−	+	−
8	Female	0.0	1.0	Japanese	CFTR-dele 16-17a-17b	TG12T7	Japanese	CFTR-dele 16-17a-17b	TG12T7	150	PI	+		+	+	+	−	−
9	Male	0.0	0.9	Japanese	CFTR-dele 16-17a-17b	TG12T7	Japanese	Deletion of exon1 in CFTR transcript	TG12T7	127	PI (0)	+	85.9	+	+	−	+	−
10	Female	1.0	3.3	Azerbaijani	F508del	TG10T9	Azerbaijani	182delT	TG10T7	62	PI	+	90.8	−	−	−	−	−
11	Female	4.6	4.8	Canadian	F508del	TG10T9	Japanese	CFTR-dele 16-17a-17b	TG12T7	66	PI (0)	+	32.5	+	−	−	−	−
12	Male	0.0	0.8	Filipino	R1066C	TG11T7	Filipino	R1066C	TG11T7	150	PI (0)	−		−	+	−	−	−
13	Female	0.4	10.7	Japanese	Y517H	TG11T7	Japanese	1540del10	TG11T7	117	PI (4)	+	89.0	−	−	−	+	−
14	Male	3.0	3.9	Japanese	CFTR-dele 16-17a-17b	TG12T7	Japanese	ND		150	PI (16)	+		+	−	+	−	Lung
15	Female	10.0	28.6	Japanese	R347H	TG11T7	Japanese	CFTR-dele 16-17a-17b	TG12T7	60	PS (910)	+	38.5	−	−	−	−	−
16	Male	0.4	0.6	Japanese	H1085R	TG12T7	Japanese	Y563H	TG12T7	110	PI (2)	+		+	−	+	−	−
17	Male	0.1	0.4	Japanese	Q1042fsX	TG12T7	European	F508del	TG10T9	150	PI	+		+	−	−	+	−
18	Female	1.8	3.4	Japanese	H1085R	TG12T7	Japanese	L441P	TG11T7	110	PI (70)	+	79.3	+	−	−	−	−
19	Male	6.6	6.6	Japanese	3499 + 56 T > C	TG11T7	Japanese	ND	TG12T7	75	PS (852)	+	22.3	+	−	−	−	−
20	Male	0.5	0.9	Japanese	CFTR-dele 16-17a-17b	TG12T7	Japanese	CFTRdele 2-3	TG11T7	83	PI	+	84.3	+	+	−	−	−
21	Female	10.0	16.0	Japanese	R75X	TG12T7	Japanese	R347H	TG11T7	27	PS (493)	+	28.0	−	−	−	−	−
22	Male	0.1	1.0	Pakistani	F508del	TG10T9	Pakistani	F508del	TG10T9	98	PI (1)	+		−	−	+	−	−
23	Male	0.8	0.9	Japanese	CFTR-dele 16-17a-17b	TG12T7	Japanese	CFTR-dele 16-17a-17b	TG11T7	110	PI (0)	+		−	−	+	−	−
24	Male	0.4	0.4	Sri Lankan	E474X	TG11T9	Sri Lankan	I1315fsX	TG11T7			+		−		−	−	−
25	Male	0.9	5.4	Japanese	CFTR-dele 16-17a-17b	TG12T7	Japanese	876-3 C > G	TG12T7	122	PI	+		−	−	−	+	−
26	Male	0.0	10.3	Japanese	E585X	TG11T7	Japanese	CFTR-dele 16-17a-17b	TG12T7	139	PI (5)	+	88.0	−	+	+	−	−
27	Female	0.0	0.1	Pakistani	F508del	TG10T9	Pakistani	F508del	TG10T9		PI (2)	+		−	−	−	−	−
28	Female	4.3	4.3	Japanese	CFTR-dele 16-17a-17b	TG12T7	Japanese	CFTR-dele 16-17a-17b	TG11T7		PI	−	96.6	−	−	−	−	−
29	Female	0.1	5.0	Japanese	CFTR-dele 16-17a-17b	TG12T7	Japanese	H1085R	TG12T7	119	PI (5)	+		+	−	−	−	−
30	Male	9.8	10.5	Japanese	CFTR-dele 16-17a-17b	TG12T7	Japanese	CFTRdele promoter	TG12T7	120	PI (3)	+	80.3	−	−	+	−	−
31	Male	0.0	0.7	Egyptian	N1303K	TG11T7	Moroccan	Q1352X	TG11T7		PI (1)	+		−	−	−	−	−
32	Female	0.4	0.7	Japanese	CFTR-dele 16-17a-17b	TG12T7	Japanese	CFTRdele promoter	TG12T7	126	PI (3)	−		−	−	−	−	−
33	Male	0.4	11.4	Japanese	G934S	TG12T7	Japanese	ND	TG12T7	106	PS (204)	+	48.0	+	−	−	−	−
34	Male	1.0	2.5	Japanese	3121-2A- > G	TG12T7	Japanese	3121-2A- > G	TG12T7		PI	+		+	−	−	−	−
35	Female	0.0	0.2	Pakistani	F508del	TG10T9	Pakistani	F508del	TG10T9		PI	−		−	−	−	−	−
36	Female	15.1	15.2	Japanese	L1156F	TG11T7	Japanese	R352W	TG11T7	63	PS (1,100)	+	86.5	−	−	−	−	−

Table 2. continued

Patient	Gender	Age at onset	Age at diagnosis	Allele-1			Allele-2			Sweat Cl	PS/PI (Fecal elastase)	Chronic sinopulmonary disease	% FEV_1	Pseudomonas aeruginosa infection	Meconium ileus	Electrolyte imbalance	CFLD	Transplant
				Ethnicity/ Country of origin	*CFTR* variant	(TG) mTn	Ethnicity/ Country of origin	*CFTR* variant	(TG) mTn									
37	Female	0.0	0.2	Japanese	H1085R	TG12T7	Japanese	CFTR-dele 16-17a-17b	TG12T7	113	PI (75)	–		–	+	+	–	–
38	Female	10.4	18.5	Japanese	ND	TG12T7	Japanese	L1156F	TG11T7	64	PS (622)	+	63.6	–	+	–	–	–
39	Female	3.4	13.7	Japanese	CFTR-dele 16-17a-17b	TG12T7	Japanese	R347H	TG11T7	105	PS (926)	+	99.2	–	–	–	–	–
40	Male	0.0	0.5	Japanese	405 + 1G- > A	TG12T7	Japanese	CFTRdele 20	TG12T7	150	PI (0)	+		+	+	+	+	–
41	Female	0.4	0.4	Japanese	L812fsX		Japanese	CFTR-dele 16-17a-17b		150	PI (0)	–		–	+	–	+	–
42	Male	4.5	5.0	Paraguayan	F508del	TG10T9	Filipino	1949del84	TG11T7		PI (0)	+		+	–	–	+	–
43	Female	0.0	5.8	Japanese	CFTR-dele 16-17a-17b	TG12T7	Japanese	3272-3 C > G	TG11T7		PI (0)	+		–	+	–	+	–
44	Female	0.0	0.2	Japanese	3121-2A- > G	TG12T7	Japanese	3121-2A- > G	TG12T7			–		–	–	–	+	–
45	Male	15.0	31.0	European	F508del	TG10T9	Turk	5 T	TG12T5		PS	–		–	–	–	–	–
46	Male	19.7	20.0	Japanese	I148F	TG11T7	Japanese	CFTR-dele 16-17a-17b	TG12T7		PS	+		–	–	–	–	–

ND Not detected
Patient No. 45 had congenital bilateral absence of the vas deferens

It has long been known that CF is rare in Asians. For example, birth prevalence of CF in Asian-Americans was estimated to be 1:35,100 (ref. [29]). Incidence of CF in Japan was previously estimated to be 3.1 per million live births by the analysis of Japanese Vital Statistics data [30]. A recent analysis of CF registry Japan data revealed that the incidence of CF in Japan is approximately 1 in 590,000 live births [3]. Literatures from China, Korea, and Taiwan suggest that CF is quite rare in East Asia [4–6].

Japan's Newborn Mass Screening does not include cystic fibrosis due to the rarity of CF in Japan. Five out of 46 definitive CF patients were diagnosed by *CFTR* variant testing before symptoms became apparent (Table 2), while over 60% of the patients in the United States are now detected by newborn screening (patient registry 2020 annual data report by the CF Foundation). While the median age of onset is 0.3 years, the median age of diagnosis is 2.5 years in Japan. The time lag is probably due to the rarity of CF in Japan.

Only a few studies have examined the impact of ethnicity on the prognosis and phenotype of CF [12, 13]. During the period August 2011~January 2013, 3 common drugs for CF (dornase alfa inhalation solution, tobramycin inhalation solution, and pancrelipase) became available in Japan. After that, the median survival of CF patients in Japan has been extended for 6.4 years to 25.2 years. This extension of survival while partly due to increased CF drug availability, is more likely due to an increased recognition of CF in Japan and increased diagnosis of patients with mild symptoms. However, the survival time is still much shorter compared to European CF patients (~40 years) [31]. Poor nutritional condition of Japanese patients with CF is demonstrated in the present study, which may cause the worse prognosis. The mean value of BMI %ile was 30.3%ile in definite CF patients aged < 18 years whose *CFTR* genotypes are known (Fig. 1A). BMI %ile of the patients with 2 East Asian/Japanese-type alleles (Japanese-Japanese: both parents are Japanese) was even lower (mean = 25.5%ile, Fig. 1C). The values are much lower compared to the mean BMI %ile (61.3%ile) of the patients in the United States (patient registry 2020 annual data). A previous study also reported lower BMI in Asian CF patients who lived in the UK [12]. The cause of poor nutritional condition of CF patients in Japan is not clear at this time.

Clinical phenotype of CF patients in Japan is characterized by more common meconium ileus and electrolyte imbalance (Table 1). Meconium ileus is more common (36%) in CF patients in Japan compared to classic CF patients in Europeans (15–20%) [31]. Children with CF have a risk of developing electrolyte imbalance due to excessive loss of Na^+ and Cl^- through sweat. In cases of extreme electrolyte loss, pseudo-Bartter syndrome may occur. There is no data on the incidence of electrolyte imbalance/pseudo-Bartter syndrome in Northern Europe and an association with warm climates has been reported [32, 33]. Electrolyte imbalance was frequently (21%) found in CF patients in Japan (Table 1) and pseudo-Bartter syndrome was reported in 3 patients. There was no significant relationship between electrolyte imbalance and CFTR genotype (Table 4).

It has long been known that the spectrum of *CFTR* variants is remarkably different among ethnic groups [34]. The present study clearly demonstrates that the spectrum of CF-causing *CFTR* variants in alleles of East Asian/Japanese ancestry is entirely different from that in alleles of European ancestry. European-type variants (such as F508del) were never detected in East Asian/Japanese alleles. Except for CFTR-dele16-17a-17b, most of the other East Asian/Japanese-type *CFTR* variants were novel or very rare. No pathogenic variants were detected in 8 (11%) of the East Asian/Japanese-type alleles (Table 2). A study of nonwhite CF patients in the United States demonstrated that fewer than 2 CFTR variants were found in 27.9% of patients [35], which is comparable to the proportion in the present study (7 in 34 Japanese-Japanese patients). Pathogenic variants in the regulatory elements or introns are probably present in many of these cases. The analysis

Table 3. Pathogenic *CFTR* variants in definite CF patients since 2007

Variant cDNA name (gDNA name)	rs ID (Variation ID)	Number of alleles	Ethnicity	Variant protein name	Variant legacy name	Location	Pathological significance (mutation class)	Reference
c.2908+1085_3367+260del	(690360)	24	East Asian/ Japanese	p.Gly970_Thr1122del	CFTR-dele 16-17a-17b	Intron 17~Intron 20	CF-causing (II)	CFMD, CFTR2, ref. [17], ref. [37]
c.1521_1523delCTT	rs113993960	11	European	p.Phe508del	F508del	Exon 11	CF-causing (II)	CFMD, CFTR2
c.1322 T > C	rs397508188	4	East Asian/ Japanese	p.Leu441Pro	L441P	Exon 10	CF-causing (II)	CFMD, ref. [38]
c.2989-2 A > G	rs193922515	4	East Asian/ Japanese	——	3121-2A- > G	Intron 18	CF-causing (V)	CFMD, CFTR2
c.3254 A > G	rs79635528	4	East Asian/ Japanese	p.His1085Arg	H1085R	Exon 20	CF-causing (II)	CFMD, ref. [39]
c.1040 G > A	rs77932196	3	East Asian/ Japanese?	p.Arg347His	R347H	Exon 8	CF-causing (III)	CFMD, CFTR2, ref. [40]
(g.117,361,112_117,498,678del)	——	2	East Asian/ Japanese	——	CFTRdele promoter	ASZ1 Exon 13 ~ CFTR Intron 1	CF-causing (I)	ref. [24]
c.223 C > T	rs121908749	2	East Asian/ Japanese?	p.Arg75Ter	R75X	Exon 3	CF-causing (I)	CFMD, CFTR2
c.3196 C > T	rs78194216	2	European	p.Arg1066Cys	R1066C	Exon 20	CF-causing (II)	CFMD, CFTR2, ref. [41]
c.3468 G > T	rs139729994	2	East Asian/ Japanese	p.Leu1156Phe	L1156F	Exon 21	Pathogenic	CFMD, ref. [8]
c.50delT	rs397508714	1	European	p.Phe17SerfsTer8	182delT	Exon 1	CF-causing (I)	CFMD, CFTR2
c.54-1760_274-10222del	——	1	East Asian/ Japanese	——	CFTRdele 2-3	Intron 1~Intron 3	CF-causing (I)	——
——	——	1	East Asian/ Japanese	——	Deletion of exon 1 in CFTR transcript	——	CF-causing (V)	ref. [17]
c.253 G > A	——	1	East Asian/ Japanese	p.Gly85Arg	G85R	Exon 3	Pathogenic	VarSome
c.273+1 G > A	rs121908791	1	East Asian/ Japanese	——	405 + 1G- > A	Intron 3	CF-causing (I)	CFMD, CFTR2
c.442 A > T	——	1	East Asian/ Japanese	p.Ile148Phe	I148F	Exon 4	Likely pathogenic	VarSome
c.650 A > G	rs121909046	1	East Asian/ Japanese	p.Glu217Gly	E217G	Exon 6	Pathogenic	CFMD, ref. [10]
c.744-3 C > G	——	1	East Asian/ Japanese	——	876-3 C > G	Intron 6	CF-causing (V)	——
c.1054 C > T	rs193922497	1	East Asian/ Japanese	p.Arg352Trp	R352W	Exon 8	Pathogenic	CFMD, CFTR2
c.1210-12 T[5]	rs1805177	1	European	——	5 T	Intron 9	CF-causing (V)	CFMD, CFTR2

Table 3. continued

Variant cDNA name (gDNA name)	rs ID (Variation ID)	Number of alleles	Ethnicity	Variant protein name	Variant legacy name	Location	Pathological significance (mutation class)	Reference
c.1408_1417delATGATTATGG	rs397508204	1	East Asian/ Japanese	p.Met470GlufsTer54	1540del10	Exon 11	CF-causing (I)	CFMD
c.1420 G > T	rs756206533	1	European	p.Glu474Ter	E474X	Exon 11	CF-causing (I)	ClinVar
c.1477_1478delCA	rs121908775	1	European	p.Gln493ValfsTer10	1609delCA	Exon 11	CF-causing (I)	CFMD, CFTR2
c.1549 T > C	——	1	East Asian/ Japanese	p.Tyr517His	Y517H	Exon 11	Likely pathogenic	VarSome
c.1624G > T	rs113993959	1	European	p.Gly542Ter	G542X	Exon 11	CF-causing (I)	CFMD, CFTR2
c.1687T > C	rs121909006	1	East Asian/ Japanese	p.Tyr563His	Y563H	Exon 13	Pathogenic	ClinVar
c.1753G > T	rs397508296	1	East Asian/ Japanese	p.Glu585Ter	E585X	Exon 13	CF-causing (I)	CFMD, CFTR2
c.1820_1903del	rs121908777	1	European	p.Met607_Gln634del	1949del84	Exon 14	CF-causing	CFMD, CFTR2
c.2433_2437delinsATA	——	1	East Asian/ Japanese	p.Leu812TyrfsTer10	L812fsX	Exon 14	CF-causing (I)	CFMD
c.2800 G > A	rs750655055	1	East Asian/ Japanese	p.Gly934Ser	G934S	Exon 17	Uncertain significance	ClinVar
c.3123_3124insA	——	1	East Asian/ Japanese	p.Gln1042fsTer	Q1042fsX	Exon 19	CF-causing (I)	——
c.3140-3 C > G	——	1	East Asian/ Japanese	——	3272-3 C > G	Intron19	CF-causing (V)	——
c.3257 C > T	rs77958296	1	East Asian/ Japanese	p.Thr1086Ile	T1086I	Exon 20	Likely pathogenic/ Uncertain significance	CFMD, ClinVar, Varsome
c.3367+56 T > C	——	1	East Asian/ Japanese	——	3499 + 56 T > C	Intron 20	Likely benign	VarSome
c.3717+3170_3874-4971del	——	1	East Asian/ Japanese	p.Val1240_Gln1291del	CFTRdele 20	Intron 22~Intron 23	CF-causing	——
c.3909 C > G	rs80034486	1	European	p.Asn1303Lys	N1303K	Exon 24	CF-causing (II)	CFMD, CFTR2, ref. [42]
c.3944_3951delTATGGAAA	rs754392413	1	European	p.Ile1315fsTer	I1315fsX	Exon 24	CF-causing (I)	ClinVar
c.4054 C > T	rs751098333	1	European	p.Gln1352Ter	Q1352X	Exon 25	CF-causing (I)	ClinVar

Reference transcript number: NM_00492.4
Human genome reference sequence: GRCh38/hg38

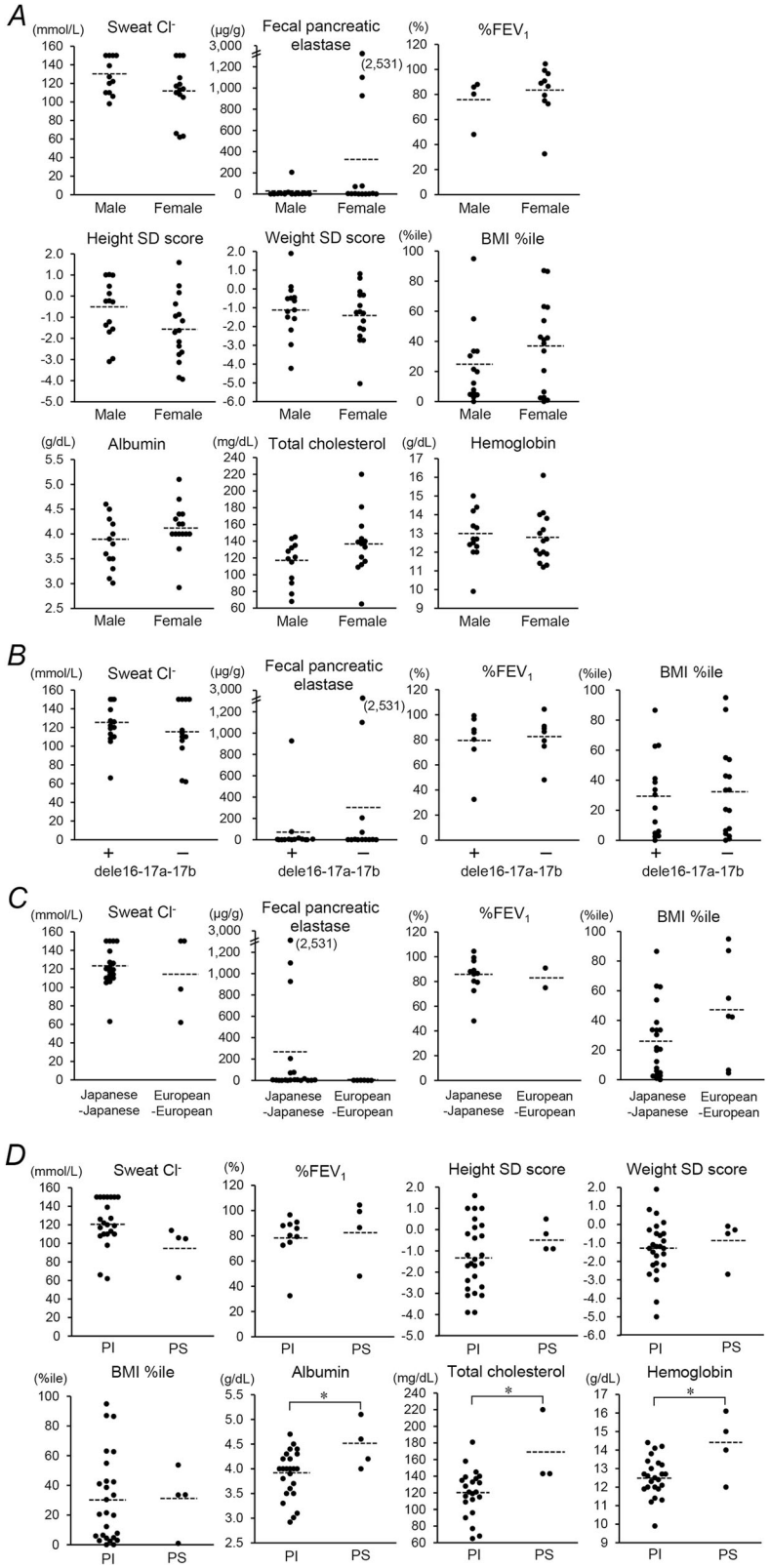

Fig. 1 Phenotype of cystic fibrosis patients in Japan. **A** Sweat Cl^-, fecal pancreatic elastase, $\%FEV_1$, SD scores of height and weight, BMI percentile (%ile), serum levels of albumin and total cholesterol, and blood hemoglobin of definite patients of cystic fibrosis aged < 18 years ($n = 30$). **B** Sweat Cl^-, fecal pancreatic elastase, $\%FEV_1$, and BMI %ile in patients who have at least one CFTR-dele16-17a-17b (dele16-17a-17b +, $n = 14$) and patients those who do not have CFTR-dele16-17a-17b (dele16-17a-17b –, $n = 16$). **C** Sweat Cl^-, fecal pancreatic elastase, $\%FEV_1$, and BMI %ile in patients with 2 East Asian/Japanese-type alleles (Japanese-Japanese, $n = 21$) and patients with 2 European-type alleles (European-European, $n = 7$). **D** Sweat Cl^-, $\%FEV_1$, BMI %ile, serum levels of albumin and total cholesterol, and blood hemoglobin in PI ($n = 26$) and PS ($n = 4$) patients. Dotted lines indicate averages

Table 4. Genotype-phenotype relationship and impact of ethnicity

		CFTR-dele 16-17a-17b		
		+	–	
Meconium ileus	+	9	5	NS
	–	12	20	
CF-associated liver disease	+	4	7	NS
	–	17	18	
Pancreatic exocrine function	PI	17	15	NS
	PS	3	8	
Electrolyte imbalance	+	8	4	NS
	–	13	21	
		Japanese-Japanese	European-European	
Meconium ileus	+	13	1	NS
	–	21	9	
CF-associated liver disease	+	8	2	NS
	–	26	8	
Pancreatic exocrine function	PI	22	8	NS
	PS	10	1	
Electrolyte imbalance	+	11	1	NS
	–	23	9	

Data are presented as numbers of patients. *NS* not significant

of CFTR transcripts may be useful to provide molecular evidence of CFTR dysfunction [17, 36].

Four types of large deletions were found in East Asian/Japanese-type CF alleles. The frequency is 40% (28/70 East Asian/Japanese-type alleles, Table 3) which is much higher than the frequency of copy-number variant in CF patients in Europeans (< 5%) [31]. While the CF Foundation patient registry previously documented that *CFTR* genomic rearrangements occurred most frequently in Asians; 2.4% of all identified alleles [35], the present study demonstrates that the frequency of *CFTR* genomic rearrangements in East Asian CF patients is much higher than previously thought.

CFTR-dele16-17a-17b was detected in one third of East Asian/Japanese CF alleles (Table 3). When CFTR-dele16-17a-17b (p.Gly970_Thr1122del) was transiently expressed in Chinese hamster ovary (CHO) cells, Δ(G970-T1122)-CFTR protein was synthesized but distributed over the intracellular compartments and not detected in the plasma membrane, thus classified to class II [37]. CFTR-dele16-17a-17b was also detected in CF patients in Korea [5] and China [4]. Whereas CFTR-dele16-17a-17b was detected in 6 out of 12 CF alleles in Korea [5], it was detected in only 1 out of 164 CF alleles in China [4]. Thus CFTR-dele16-17a-17b is probably a Japanese- and Korean-specific variant.

We acknowledge the present study has limitations. We have not performed ethnicity adjustment for $\%FEV_1$ and BMI %ile. Although patients who were not born in Japan are excluded, $\%FEV_1$ and BMI %ile of patients whose parents are not of Japanese origin, may not be accurately evaluated.

In conclusion, while CF is rare in Japan, we report that the spectrum of *CFTR* variants in East Asian/Japanese CF alleles is entirely different from that in European CF alleles. Moreover, whereas clinical phenotype of Japanese CF patients is similar to European CF patients, the prognosis of CF patients in Japan is worse.

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ACKNOWLEDGEMENTS

We thank Drs. Ito S, Ichinoi N, Wada Y, Sakamoto O, Sanada Y, Harada Y, Shoji S, Hoshikawa Y, Kanda K, Kando N, Shimizu M, Ichihara T, Endo A, Mizuno Y, Obata M, Maruyama S, Baba Y, Yanagimoto K, Arai K, Kojima D, Sato Y, Itoh K, Takahara T, Harada T, Ishii H, Kushima H, Fukuda Y, Tanoue K, Kawakita R, Murakami Y, Asao T, Kitabayashi T, Azuma A, Usuki J, Koga H, Hagio Y, Fukasawa M, Fukaya S, Ito R, Abukawa D, Hoshi Y, Umetsu S, Ishii M, Kojiro M, Kawase M, Tabata K, Ito T, Arakaki Y, Yokoyama N, Mitani Y, Fukumori T, Ishikawa S, Wada T, Nagai Y, Shiona S, Sakata Y, Yamasaki K, Igata F, and Ishii H for providing clinical data of CF patients. We thank Dr. Stewart AK for English editing of the manuscript.

FUNDING

This work was supported by grants from the Japan Society for the Promotion of Science, the Japanese study group for pediatric rare and intractable hepato-biliary-pancreatic diseases provided by the Ministry of Health, Labour, and Welfare of Japan, and scholarship donations by Chugai Pharmaceutical Co. Ltd.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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