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Spectrum of Mutations in the *CFTR* Gene of Patients with Classical and Atypical Forms of Cystic Fibrosis from Southwestern Sweden: Identification of 12 Novel Mutations

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ABSTRACT

Cystic fibrosis (CF) is caused by mutations in the *CFTR* gene. The spectrum of *CFTR* mutations varies between populations and depends on different factors, such as ethnic background and geographical location. The extensive *CFTR* mutation screening of 129 patients with classical or atypical CF from the south-western region of Sweden revealed the presence of 37 *CFTR* mutations, including 12 novel alleles. The overall mutation detection rate in this study population was 92%, the highest among all tested regions in Sweden. Eight mutations with a frequency above 1% (ΔF508, 394delTT, R117C, 3659delC, E60X, 1112delT, R764X, and 621 + 1G → T) accounted for 78% of CF chromosomes and have been recommended for inclusion in the CFTR mutation screening panel for molecular diagnosis of CF in this region. The multiple occurrence of specific *CFTR* alleles less common than the predominant ΔF508 mutation (394delTT, R117C, 3659delC) allowed for genotype–phenotype comparisons and revealed consistent relationships between these mutations and disease severity.

INTRODUCTION

CYSTIC FIBROSIS (CF) is one of the most common autosomal recessive disorders in Caucasians (Welsch *et al.*, 1994). The estimated incidence of CF in Sweden is ~1:6000 of newborns, with a carrier frequency of 1:50–60 (Selander, 1962). CF, in its classic form, is characterized by chronic pulmonary disease, pancreatic insufficiency, and elevated concentrations of sweat electrolytes. CF is caused by mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene, which is located on the long arm of chromosome 7. The gene encodes a transmembrane protein apically expressed in various epithelial cells and functioning as a cAMP regulated chloride channel (Rommens *et al.*, 1989; Riordan *et al.*, 1989). Since the initial characterization of the *CFTR* gene and description of the most common mutation, ΔF508, over 1000 other mutations have been reported worldwide (CF Genetic Analysis Consortium Web site; November 2001: http//www.genet.sickkids.on.ca/cftr/).

CFTR mutations are associated with various molecular mechanisms that may determine clinical severity of CF (Tsui, 1992; Welsh and Smith, 1993; Zielenski and Tsui, 1995). Generally, mutations producing *CFTR* variants with residual chloride channel function tend to be associated with milder CF presentations, although the particular phenotypic effect varies between affected organs (Zielenski, 2000). The strongest correlation between *CFTR* genotype and severity of cystic fibrosis symptoms has been observed for the pancreatic exocrine function (Kristidis, 1992). In contrast, the pulmonary component of the CF phenotype shows a rather poor correlation with the *CFTR* genotype, probably due to the strong influence of environmental and secondary genetic factors (Zielenski, 2000).

The spectrum of *CFTR* mutations is population-specific and varies between different populations and depends on ethnic background and geographical location (The Cystic Fibrosis Genetic Analysis Consortium, 1994). Analysis of the composition and frequency of *CFTR* mutations in a particular population

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TABLE 1. MUTATIONS IDENTIFIED IN 258 CHROMOSOMES IN THE CF POPULATION ATTENDING THE SOUTH-WESTERN SWEDISH CF CENTRE

Mutation	*Location in the gene, exon*	*Number of mutations*	*Frequency of mutation (%)*	*Homozygotes*	*Heterozygotes*
ΔF508	10	161	62.4	56	49
394delTT	3	13	5.0	3	7
R117C	4	7	2.7		7
3659delC	19	5	1.9		5
E60X	3	4	1.6		4
1112delT	7	4	1.6	1	2
R764X	13	4	1.6	1	2
621 + 1G → T	4	3	1.2		3
G551D	11	2	0.8		2
I506L	10	2	0.8		2
N1088D (R75Q)	17b	2	0.8		2
Q1238X	19	2	0.8		2
R117H (IVS8-5T)	4	2	0.8		2
V603F (IVS8-5T)	13	2	0.8		2
1716G → A	10	2	0.8		2
R75Q	3	2	0.8		2
R533X	11	1	0.4		1
−329A → G	Promoter	1	0.4		1
297-3 C → A	2	1	0.4		1
Y161D	4	1	0.4		1
994del9	Exon/intron 6b	1	0.4		1
1154insTC	7	1	0.4		1
W361R	7	1	0.4		1
T338I	7	1	0.4		1
1249-5A → G	Intron 7	1	0.4		1
1717-2A → G	Intron 10	1	0.4		1
R560T	11	1	0.4		1
E1401X	23	1	0.4		1
3126del4	17a	1	0.4		1
S945L	15	1	0.4		1
R668C	13	1	0.4		1
2622 + 2del6	Intron 13	1	0.4		1
R1162Q	Exon 19	1	0.4		1
3849 + 10kbC → T	Intron 19	1	0.4		1
R74W	Exon 3	1	0.4		1
−363C → T	Promoter	1	0.4		1
IVS8-5T[a]	Intron 8	1	0.4		1
Unidentified		20	7.8		
Total		258	100	61	116

The new mutations are displayed in bold.
[a] Splicing site variation in the T-tract of intron 8.
Note: The patients carrying the 1112delT allele were related (siblings).

provides important information, both for the understanding of mechanisms correlating genotype and phenotype, as well as for the establishment of population-based molecular diagnostics.

Here we present the results of an extensive analysis of the *CFTR* mutation spectrum in patients from the southwestern Swedish CF Centre.

MATERIALS AND METHODS

Patients

All patients attending the Goteborg CF Centre (in Queen Silvia Children's Hospital) during the period of 1995–1999 were screened for mutations in the *CFTR* gene. Out of 129 patients investigated, 125 (65 male and 60 female) presented with classical clinical manifestations in conjunction with an elevated sweat test (Gibson and Cooke, 1959). Four patients with non-diagnostic sweat test results were included because CF was suspected due to pulmonary, gastrointestinal, and/or sinusitis symptoms.

Clinical evaluation of the patients included age at diagnosis and disease verification by X ray, spirometry, and regional lung function tests with Tc^{99}. *Pseudomonas aeruginosa* colonization was defined as at least three consecutive sputum cultures during the last 6 months and/or positive serum antibodies against Pseudomonas antigens (Hollsing *et al.*, 1987). Pancreatic status was evaluated by determination of faecal chymotrypsin or

elastase activities, a fat tolerance test, and/or measurements of pancreatic enzyme activity in duodenal juice after stimulation with secretin and pancreozymin. Pancreatic sufficiency was defined as a clinical history of normal growth, absence of gastrointestinal symptoms and fat malabsorption, and no need for pancreatic enzyme replacement therapy.

Screening for CFTR mutations

Genomic DNA was obtained from peripheral blood of patients using the standard protocols. All patients were first analyzed for the most common *CFTR* mutation, ΔF508, and for the second most common mutation in Scandinavia, 394delTT. ΔF508 was detected by allele-specific PCR (Ballabio *et al.*, 1990) and 394delTT by heteroduplex analysis on polyacrylamide gels using the Pharmacia Phast System™. Patients negative for one or two of these mutations were then subjected to mutation screening using polymerase chain reaction (PCR) and multiplex heteroduplex (mHET) analysis on the Hydrolink gel matrix (Zielenski *et al.*, in press) and direct sequencing using the Thermo Sequenase radiolabeled terminator cycle sequencing kit (USB). In addition, the thymidine tract (T-tract) in the acceptor splice site in intron 8 was evaluated to detect the RNA splice variant IVS8-5T (Mak *et al.*, 1997).

RESULTS

Spectrum of CFTR mutations

The cumulative results of *CFTR* mutation screening in 129 CF patients (258 chromosomes) are presented in Table 1. ΔF08 deletion, the most common mutation in Caucasian populations, was found in 62.4% (161/258) of the CF alleles. Of the remaining CF alleles, 77 (29.8%) carried 36 different *CFTR* mutants/variants (Table 1). The second most common mutation, the deletion 394delTT, characteristic for Nordic (Scandinavian) population accounted for 5% of the CF alleles. Two other mutations with frequencies exceeding 2% were R117C and 3659delC. Twenty mutations were carried on a single CF chromosome. The margin of CF chromosomes with unidentified mutations using this methodology was 7.8% (20/258) even though the diagnosis of CF was clear (with the exception of 4 patients) from the clinical point of view, including pathological sweat tests. The overall efficiency of *CFTR* mutation screening in this study population was 92.2%.

At the genotype level, 43% (56/129) of the patients were homozygous for the ΔF508 mutation and 38% (39/129) were compound heterozygous for the ΔF508 allele and another mutation. In addition, 5 patients were homozygous for other mutations; three for 394delTT, and one each for 1112delT and R764X (Table 1 and 2). Among the most common non-Δ508 alleles, three (394del TT, R117C, and 3659delC) were detected in the context of full *CFTR* genotypes in 22 CF patients (Table 2).

The availability of clinical data for the patients allowed for genotype–phenotype correlations for the latter three alleles. The 394delTT mutation ($n = 13$) was associated with a very severe CF presentation, which includes high sweat chloride concentration, pancreatic insufficiency (PI), moderate to severe lung disease, and high rate of liver disease (~40%). In contrast, patients ($n = 7$) with the R117C allele (all paired with the ΔF508 deletion) tended to present with a mild form of CF [pancreatic sufficiency (PS), good pulmonary status]. As expected, patients with the 3659delC and ΔF508 mutations presented with severe CF. One patient with the 3659delC allele and a mild mutation on the second chromosome (I506L), presented with a mild form of CF (Table 2).

TABLE 2. CLINICAL PROFILES OF THE PATIENTS WITH MOST COMMON *CFTR* MUTATIONS (ΔF508 NOT INCLUDED)

		Patient clinical data					
Mutation/ genotype	n	*AD/yr median (range)*	*PI/PS*	*Lung involvement*	*MI*	*Cl^- (mmol/liter) median (range)*	*Other*
394delTT	10	1.5 (0.1–12.2)	13/0	10/10	1/10	109 (90–140)	
394delTT/ΔF508	7	1.5 (0.1–12.2)	7/0		1/7	110 (90–140)	4 with liver disease of whom 1 died (*B. cepacia* syndrome)
394delTT/394delTT	3	1.5 (0.8–4.2)	3/0		0/3	102 (100–118)	
R117C	7	5.5 (2.5–18)	0/7	1/7	0/7	85 (71–100)	
R117C/ΔF508	7	5.5 (2.5–18)	0/7		—	85 (71–100)	
3659delC	5	0.8 (0.3–29)	4/1[a]	5/5	0/5	106 (80–116)	
3659delC/ΔF508	4	0.6 (0.3–8.0)	4/0		0/4	107 (100–116)	2 double lung transplanted (34, 28 years), of whom 1 had diabetes mellitus
3659delC/I506L	1	29	0/1*		0/1	80	

AD, Age at diagnosis in year; PS/PI, pancreatic status (sufficiency/insufficiency); Lung, lung disease; MI, meconium ileus; Cl^-, sweat chloride conc.; Other, other symptoms.

[a]The second mutation (I507L) is mild; therefore, the patient is PS.

TABLE 3. CLINICAL DATA FOR THE CF PATIENTS CARRYING NEW MUTATIONS

Mutations	*Age (years) at diagnosis*	*PI or PS*[b]	*Lung disease (severity)*	*Sweat Cl (mmol/liter)*	*Additional symptoms*
Frameshift					
1112delT/1112delT	4	PI	+++	110	
1112delT/ΔF508	0.3	PI	+++	112	
1112delT/ΔF508	0.2[a]	PI	+++	110	
3126del4/E60X	2	PI	++	130	
994del9/ΔF508	0.08	PI	−	120	Meconium ileus
RNA splice					
297-3C → A/ΔF508	0.3	PI	+	120	
2622 + 2del6/ΔF508	0.25	PI	+++	100	
Nonsense					
E1401X/unknown	6	PS	−	52	Poor growth, fat malabsorption, abnormal electrophysiological response in the intestinal mucosal biopsy
Missense					
V603F, IVS8-5T/ΔF508	2	PI	+	101	
N1088D, R75Q/ΔF508	4[a]	PS	−	78	
N1088D, R75Q/ΔF508	2	PS	−	75	
Y161D/ΔF508	0.4	PI	+	83	Malabsorption
I506L/ΔF508	42.5	PS	+++	103	
I506L/3659delC	30	PS	+++	80	
R1162Q/unknown	nv[c]	PS	+	6	Frequent pneumonias
V603F, IVS8-5T/unknown	nv[c]	PS	(+)	24	Sinusitis, severe recurrent hypoglycemia, nasal polyps, abdominal pain
Promoter?					
−329A → G/1716G → A	nv[c]	PS	(+)	50	Recurrent abdominal pain, nasal polyps
−363C → T/unknown	nv[c]	PS	−	43	Abdominal pain

[a]Diagnosis was obtained due to sick sibling.
[b]PI, pancreatic insufficiency; PS, pancreatic sufficiency.
[c]nv, not verified.

Besides the *CFTR* alleles previously reported by others, 12 novel mutations/variants were found in 17 patients. They represent different types of mutations (missense, nonsense, frameshift, and RNA splice site), are located in various exon and intron regions, and are associated with a spectrum of clinical phenotypes (Table 3). Preliminary information on all new mutations was submitted to the CF Genetic Analysis Consortium (*http://www.genet.sickkids.on.ca/cftr/*).

Novel CFTR mutant variants

RNA splice mutations: **297-3C → A.** This mutation was caused by a transversion C to A in intron 2 at nucleotide position 297-3 of the *CFTR* gene. The change is located in the acceptor splice site and may potentially affect the splicing of exon 3. The 297-3C → A allele was found in one patient carrying the ΔF508 deletion on the other chromosome and presenting with mild pulmonary disease and PI (Table 3).

2622+2del6. This is a 6-nucleotide (TAGGTA) nonframeshift deletion in intron 13 starting at position 2622+2 and affecting the 5′ splice site. It was found in 1 patient with a severe form of CF and the ΔF508 deletion on the other chromosome.

Frameshift and nonsense mutations: **994del9.** Formally, it is a frameshift mutation in exon 6b; however, since it also deletes the first nucleotide (1001+1G) of the invariant 5′ splice site in intron 6b, it should be regarded as a splice site mutation. The mutation is caused by the deletion of 9 nucleotides (TTAAGACAG) between positions 994 and 1002. It was found in a young patient with meconium ileus, and PI but no symptoms of lung disease. The patient carried the ΔF508 mutation on the other chromosome.

1112delT. This frameshift mutation was found in exon 7 of the *CFTR* gene, and it is caused by deletion of a single T at the nucleotide position 1112. The allele was found in 3 patients, including one homozygous for this allele. All patients (two of

them siblings, also with the ΔF508 allele) presented with a severe form of CF. All of these patients were related. The 1112delT allele in the siblings was inherited from their mother, who is of Finnish origin.

3126del4. This frameshift mutation in exon 17a was caused by deletion of 4 nucleotides (ATTA) between positions 3126 and 3129. The allele was found in 1 patient with the E60X mutation on the other chromosome. The patient manifested with an elevated sweat chloride concentration (100 mmol/liter), PI, and moderate lung disease.

E1401X. This is a nonsense mutation in exon 23 caused by a transversion G to T at nucleotide position 4333. In consequence, it introduces a termination codon at the amino acid position 1401 of the CFTR polypeptide. Truncation of the terminal portion of CFTR (up to ~98 amino acid residues) destabilizes the complex-glycosylated form of the CFTR molecule (Haardt *et al.*, 1999) without affecting its biosynthesis, processing, and macroscopic channel function. The mutation was found in a 6-year-old patient from Lebanon, presenting with poor growth and fat malabsorption, but overall mild symptoms (sweat chloride 52 mmol/liter, no pulmonary symptoms, and PS). He had a typical for CF electrophysiological response in the intestinal mucosal biopsy (Hallberg *et al.*, 2000). Because the second mutation is unknown, the phenotypic status of the E1401X cannot be established.

Besides changing the reading frame, these frameshift mutations (994del9, 1112delT, and 3126del4) also introduce premature termination codons at or downstream of deletion points (TAG at codon 307, TAA at codon 368, and TAA at codon 99, respectively). In addition, the 994del9 mutation also affects a splice site.

Missense mutations: **Y161D.** Located in the first cytoplasmic loop (CL1) in exon 4, this missense mutation was caused by a transversion G to T at nucleotide position 613 and consequently changes of tyrosine to aspartic acid at the amino acid position 161. Mutations in the CL1 are known to alter a transition to open state of the CFTR chloride channel (Seibert *et al.*, 1997). The ΔF508 deletion was the second mutation in this patient presenting with PI and malabsorption as the main symptoms, with a mild pulmonary disease. This child's mother is from Macedonia.

V603F. This missense mutation in exon 13 (R-domain) was caused by a transversion of G to T at the position 1939 of the *CFTR* gene and, in consequence, the change of valine to phenylalanine at amino acid position 603. This allele was found in a female patient with the ΔF508 as the second allele, and the RNA splice site IVS8-5T variant co-segregating with the V603F mutation. She has PI, pulmonary symptoms, and a sweat chloride of 101 mmol/liter. The mother is of East Indian origin. Interestingly, the mother's sister carried the same mutation in conjunction with the RNA splice site IVS8-5T variant, a second mutation is as yet unidentified. She has normal sweat chloride (28 mmol/liter), mild pulmonary disease, sinusitis, and severe, recurrent hypoglycemia. Her CF diagnosis is, therefore, not verified.

I506L. A missense mutation within NBD1 (exon 10) was caused by a transversion A to C at nucleotide position 1648 leading to the conservative amino acid change of isoleucine to leucine at position 506. The mutation was found in 2 unrelated patients; 1 with the I506L/ΔF508 genotype (sweat Cl^-/103 mmol/liter, PS, and severe lung disease), and the other with the I506L/3659delC genotype (sweat Cl^- 80 mmol/liter, PS, and severe lung disease). Both were diagnosed in adulthood at 42 and 30 years of age, respectively.

R1162Q. Located in the cytoplasmic segment of CFTR between TM12 and NBD2, this missense variant is caused by a transversion G to A in exon 19 at nucleotide position 3617. It leads to a change of arginine to glutamine at amino acid position 1162. The female patient carrying this *CFTR* variant presented with frequent pneumonias, normal pancreatic function, and sweat chloride levels (6 mmol/liter). The CF diagnosis has not been verified in this patient. Although the second mutation in this patient is unknown, the R1162Q allele is expected to be very mild, because another mutation in this codon, R1162L was previously classified as a normal variant (Fanen *et al.*, 1992).

N1088D. This mutation was detected in the fourth cytoplasmic loop (CL4) of CFTR. It was caused by a transversion A to G in exon 17b at nucleotide position 3394 and consequently, replacement of asparagine by aspartic acid at amino acid position 1088. The N1088D was found in two siblings (genotype N1088D/ΔF508) with PS, and mild or no lung disease. There was a third *CFTR* variant, R75Q, found in these patients and associated with the N1088D allele.

In addition to the well-defined mutations listed above, 2 patients carried single nucleotide substitutions in the promoter region of the *CFTR* gene (positions −363C → T or −329A → G). Without functional tests, however, it is not clear if these changes are contributing to the phenotypes of these patients. One female patient (with −363C → T) presented has an atypical clinical presentation (recurrent abdominal pain, a borderline sweat chloride of 43 mmol/liter, and normal pancreatic and pulmonary functions). No other *CFTR* allele, except the intron variant 1525-60 G → A was found in this patient. The patient carrying the −329A → G mutation also had recurrent abdominal pain, a borderline sweat chloride of 50 mmol/liter, nasal polyps, PS, and slight pulmonary involvement. The other mutation in this patient was 1716G → A.

The summary of *CFTR* genotypes, consisting of the new *CFTR* alleles and the corresponding clinical data is shown in Table 3.

In our study population, there were 11 CF families with 2 affected siblings, and 1 with 3 affected children. All sibs had the same *CFTR* genotypes, four homozygotes for ΔF508, and the remainder was compound heterozygotes.

DISCUSSION

A total of 129 CF patients ascertained from CF clinics in the southwestern region of Sweden were subjected to extensive mutation screening of the entire *CFTR* gene. Effectively, the screening protocol using the mHET included 1 kb of 5′ untranslated region (UTR) region, the 27 exons with flanking introns, and some other specific regions (*e.g.*, intron 19 for the 3849 + 10 kb C → T mutation). As a result, *CFTR* mutations/variants were found in 92.2% (238/258) of the patients' chromosomes. This overall detection rate is in agreement with the method's mutation screening capacity of 95%, as estimated by the retrospective screening (Zielenski *et al.*, in press). The

TABLE 4. REGIONAL DISTRIBUTION OF *CFTR* ALLELE FREQUENCY IN SWEDEN

			CFTR alleles											
	Patient chromosomes		*ΔF508*		*394delTT*		*3659delC*		*R117C*		*Other*[e]			*All identified*
Center	n	%	n	%	n	%	n	%	n	%	n	%	*Nm*	*(%)*
Uppsala[a]	60	6.6	35	58.3	4	6.7	8	13.3	nd[c]					
Stockholm[a]	354	38.5	238	67.2	29	8.2	37	10.5	nd[c]		15	2.3	8	84.7
Lund[a]	248	26.9	179	72.1	23	9.3	7	2.8	6[d]	2.3				
Goteborg[b]	258	28	161	62.4	13	5.0	5	1.9	7	2.7	52	20.2	33	92.2
Sweden total	920	100.0	613	66.6	69	7.5	57	6.2	13	1.4	67	7.3		89.0

[a]The data for Uppsala, Stockholm, and Lund were presented according to the published report (Schaedel *et al.,* 1999).
[b]This study.
[c]nd, No data.
[d]R117C or R117H.
[e]This section contains data corresponding to other less common mutations. *n* and % refer to number and proportion of CF chromosomes carrying the other *CFTR* alleles. The "Nm" column represents the number of different *CFTR* mutant alleles identified among CF chromosomes not carrying most common mutations (ΔF508, 394delTT, 3659delC, and R117C). Results from other centers are cumulative.

screening led to the identification of 37 different *CFTR* alleles, including 12 previously unknown mutations.

The allele frequency of the most common mutation ΔF508 was 62.4%, which is significantly lower than that previously observed for the southern Swedish CF population (72%) (Kornfalt *et al*., 1992) and the Danish CF population (88%) (Schwartz *et al*., 1994). As expected, the 394delTT frameshift mutation characteristic for Nordic populations (Schwartz *et al*., 1994) was the second most common mutation in our study population, at a frequency of 5.0%. This was lower than that reported at the Lund center (9.3%). As with the southern region of Sweden (Lund), the second most common mutation (3659delC) in the eastern part of the country (Uppsala 13.3%, Stockholm 10.5%), was much less represented in our patients (1.9%) and it was surpassed by another mutation, R117C (2.7%) (Table 4).

Our study provides the most complete mutation screening data of all the populations evaluated in different regions of Sweden, with the rate of detection of 92.2% (Table 4). A large number (33) of different *CFTR* mutations/variants, each with a low frequency (20 of them occurred only once; Table 1), were detected and characterized in 20.2% of CF chromosomes (Table 4) in this population. This finding probably reflects an increasing ethnic complexity of the contemporary Swedish population. Although the spectrum of mutations from other centers is not completed with a significant number of CF chromosomes still with unidentified mutations (~15% of the total studied; Table 4), one may expect that these most likely represent rare mutations, rather than more frequent ones already reported in other centers. The differences in local mutation spectra are seen even between nondistant regions. For example, in the southern part of Sweden, which previously belonged to Denmark, only four mutations accounted for 86% of the alleles, and in the southwestern part of Sweden, which is similar in terms of surface area and population size, a much larger number, 19 mutations, accounted for the same percentage (Tables 1 and 4).

The relatively high frequency of the 394delTT (3 homozygotes) R117C and 3659delC alleles made it possible to draw genotype–phenotype correlations for patients carrying these mutations (Table 2). Patients carrying the 394delTT mutation (either homozygous for this allele, or compound heterozygotes with the ΔF508) tend to present with the severe phenotype, with symptoms including PI at an early age and lung disease with chronic or intermittent *Pseudomonas aeruginosa* colonization. One patient died at the age of 18 with the "Burkholderia cepacia syndrome." In addition, these patients had a high incidence of biochemical liver disease, the frequency of which was not significantly different from that in homozygotes for ΔF508 (36%). This is in agreement with an earlier prospective study of liver disease in Sweden where no relation could be found between genotype and liver disease (Lindblad *et al*., 1999).

The group of patients with the combination of mutations 3659delC and ΔF508 had a similar phenotype to that in patients with 394delTT with early disease diagnosis, PI, and severe lung disease. One patient with I506L and 3659delC was diagnosed at a later age and presented with PS, but severe lung disease. In the group carrying the mild R117C allele all patients were PS and diagnosed at older ages. Characteristically, all but one of these patients has normal lung function and none of them were chronically colonized with *P. aeruginosa.* Only 1 patient has slight bronchiectasis with intermittent growth of *Staphylococcus aureus.*

Beside the *CFTR* mutations reported before, 12 novel *CFTR* alleles have been found in this study population (Tables 1 and 3). The clinical data associated with these mutations are listed in Table 3. Five of these mutations belong to the class I mutations that severely affect the synthesis and/or structure of CFTR protein and are therefore associated with more severe CF phenotypes, provided that the other allele is also severe (Tsui, 1992; Zielenski and Tsui, 1995). Interestingly, one of these mutations (E1401X) is associated with a very mild CF presentation (age at diagnosis, 6 years; borderline sweat chloride levels of 52 mmol/liter, PS, and no lung symptoms). Although the second mutation in this patient has not yet been identified, there is a possibility that the CFTR carboxy-terminal truncation caused

by the nonsense mutation may produce a relatively unstable but fully functional CFTR channel that may partially account for the milder presentation (Haardt *et al.*, 1999). Frameshift and nonsense mutations belonging to this category may be put in a new class (VI) of mutations based on their distinct molecular mechanism leading to protein instability (Haardt *et al.*, 1999; Zielenski, 2000).

The remaining mutations are mainly of the missense type and probably represent different classes. Two of them (V603F and Y161D) are associated with PI, moderate-to-severe lung disease, and elevated sweat chloride levels. The remaining missense variants (I506L, N1088D, and R1162Q) can be classified as mild alleles because they are associated with a milder CF phenotype in genotypes with a severe second allele. These missense mutations are probably of the class IV or V types, which are associated with a residual *CFTR* function. Besides these missense variants, there is also splice mutation in the −3 position of the acceptor splice site in intron 2 (297-3 C → A). The nucleotide alterations in this position are less restrictive for the splice selection and recognition process and are expected to produce some amount of correctly spliced mRNA transcript (class V). Indeed, the mutation was associated with a milder CF presentation (Table 3). Finally, there were two alleles (−363C → T and −329A → G) identified in the 5′ UTR (promoter) region. The former variant was found in a patient with borderline sweat Cl^- (43 mmol/liter), recurrent abdominal pain, but no pancreatic or lung disease. Without a functional study it is impossible to verify the disease-causing status of this mutation; however a second mutant allele was not found in this patient.

Near-complete screening of a local CF population provides important information for establishing the most optimal strategies for routine screening of CF patients in this population. Our results indicate that an adjustment should be made to the panel of most common mutations used in the screening of the southwestern Swedish CF population: the addition of the R117C mutation (2.7% of CF chromosomes; Table 1). This mutation is known to be associated with a variety of clinical presentations, extending from congenital bilateral absence of the vas deferens (CBAVD) to mild CF (Massie *et al.*, 1999; Barolozzi *et al.*, 2000). The phenotypic effect of this mutation, like that of the more common R117H allele, may depend on the length of the T-tract variant in intron 8 (Massie *et al.*, 1999). Screening for this mutation will be especially relevant for the patients presenting with the atypical or milder form of CF and will require the T-tract evaluation.

As more clinical presentations resembling CF are being brought to medical attention, it has become a challenge to make a firm diagnosis of CF based on atypical clinical findings and negative or partially negative results of a genetic analysis of the *CFTR* gene. This also raises questions as to where to draw the line between CF and CF-like clinical phenotypes. One example from our study population is a patient with an atypical CF presentation (recurrent abdominal pain; borderline sweat chloride; normal pancreatic and pulmonary function). The *CFTR* mutation screening revealed the presence of a potential promoter mutation −363 C → T in the 5′ UTR region but not other well-defined *CFTR* allele. If we assume that the −363C → T variant is indeed affecting *CFTR* function, was a second mutation required to produce the clinical manifestations observed in this patient? Interestingly, the overall *CFTR* mutation detection rate (even when using efficient mutation screening methods) in patients presenting with very mild or atypical CF tends to be much lower (50–60%) than that observed in patients with classic CF (90–100%) (Zielenski, Walker, and Tsui, unpublished data). This and other observations are consistent with the idea that the carrier state under certain—yet undefined—conditions may lead to an atypical CF phenotype (Bronsveld *et al.*, 1999). One possible explanation is an impact of secondary genetic factors that may, through as yet unknown molecular mechanisms, contribute to expression of CF-like symptoms in carriers of *CFTR* mutations (Zielenski *et al.*, 2000).

Another important issue is making the diagnosis of CF in such atypical cases. Many of them would not be diagnosed as CF under currently recommended CF diagnosis criteria (Rosenstein and Cutting, 1998). This poses serious problems for physicians and genetic counselors when trying to interpret the clinical and genetic data available for a particular patient to make a definitive CF diagnosis.

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