

The I148T *CFTR* allele occurs on multiple haplotypes: A complex allele is associated with cystic fibrosis

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Purpose: To determine whether *CFTR* intragenic changes modulate the cystic fibrosis (CF) phenotype in individuals who are positive for the I148T allele. **Methods:** The *CFTR* genes from individuals who carried at least one copy of the I148T allele were analyzed for additional changes that may be acting as genetic modifiers. **Results:** Seven of eight individuals with a known or suspected diagnosis of CF who carried I148T in combination with a severe CF mutation also carried 3199del6. Eight apparently healthy adult individuals who were compound heterozygous for I148T and a severe CF mutation or homozygous for I148T did not carry the deletion ($P = 0.0014$). The I148T allele occurs on at least three haplotypes: an IVS-8 9T background, a 7T background, or a 9T + 3199del6 background. The 3199del6 allele was not identified in 386 non-CF chromosomes. **Conclusions:** It is concluded that I148T occurs on at least three haplotypes and the complex allele I148T + 9T + 3199del6 is associated with a classic CF phenotype. **Genet Med 2002;4(5):319–323.**

Key Words: cystic fibrosis, genotype, phenotype, complex alleles, *CFTR* mutations

Over the last decade, mutation analysis of the *CFTR* gene in patients with cystic fibrosis (CF) and related syndromes has identified more than 1000 mutations.¹ Some mutations may be classified as severe or mild/variable based on their effect on protein function and/or the resulting clinical phenotype. A severe mutation may result in no protein production or a protein with impaired functional activity. Individuals carrying two severe mutations often manifest the clinical signs of classic CF: progressive lung disease due to chronic infection, pancreatic exocrine insufficiency, infertility in males, and elevated sweat chloride.² Mild/variable mutations produce proteins with some residual function. When these mild/variable mutations occur in combination with a severe mutation, or second mild/variable mutation, the CF phenotype may range from typical CF to single-organ diseases, such as congenital bilateral absence of the vas deferens (CBAVD) in males and idiopathic chronic pancreatitis.^{3–6}

Genetic modifiers within the *CFTR* gene are also known to affect the expression of the disease phenotype by modulating the effect of a mutation. Most notable is the effect of the length of the intron 8 polythymidine tract (5, 7, or 9 thymidines) on

exon 9 splicing and the phenotypic expression of the R117H mutation.^{7,8} Individuals with a severe CF mutation and R117H in *cis* with 5 thymidines (5T) may have moderate (pancreatic sufficient) CF, whereas those with R117H and 7 thymidines (7T) in *cis* may be asymptomatic and have CBAVD or later-onset lung disease such as bronchiectasis.^{8,9}

Only a small number of all *CFTR* mutations result in a predictable disease phenotype. The classification is usually based on careful clinical description of patients with defined genotypes. The I148T allele has been identified in CF patients from several countries and has been characterized as a “severe” CF mutation because some of these patients had pancreatic insufficient CF.^{10–13} It accounts for 0.7% of CF chromosomes in Italian patients,¹² 0.2% in Canadian patients,¹⁴ 1.1% in US patients from the state of Maine,¹⁵ 0.08% in French patients,¹⁶ and 9.1% in French-Canadian patients.¹⁷ In addition, the substitution changes a conserved amino acid and occurs in the first cytoplasmic loop of the first membrane spanning domain (MSD1).^{18,19} Based on such data, I148T was included in the ACMG/ACOG recommended CF mutation screening panel.^{20,21}

Functional analysis of the I148T allele in transfected cell lines indicates that the protein product from this allele is fully glycosylated and biosynthetically mature. Anion conduction, as measured by iodide efflux, is normal in cells containing the I148T allele, and the cells have gating properties identical with those of wild-type cells.¹⁹ While this analysis does not rule out the possibility that the I148T allele affects other functions, such as its ability to function as a sodium channel, several major *CFTR* functions are retained.

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We now have evidence that the I148T allele is associated with variable phenotypes. First, we had previously identified apparently healthy individuals who were compound heterozygotes for $\Delta F508$ and I148T.²² Second, we observed that the frequency of I148T is significantly higher in CF carriers than CF patients.^{22,23} To determine whether additional changes within the *CFTR* gene were influencing the CF phenotype when I148T was present, we sequenced the coding regions of the *CFTR* gene in apparently healthy individuals and CF patients who were compound heterozygous or homozygous for I148T. Here we show that the variable phenotypes are due to the occurrence of an in-frame deletion in *cis* with I148T on some chromosomes.

MATERIALS AND METHODS

Patient samples

From January 1, 1998, to December 31, 2000, carrier or diagnostic testing was performed on individuals from various ethnic backgrounds primarily from the United States. No age limit was applied when determining the frequency of I148T in carriers, CF patients, or suspected CF patients. Patients designated as suspected of having CF were so designated on the basis of information provided by the physician at the time of testing. As described below, carriers, CF patients, and suspected CF patients who carried I148T and a severe mutation were tested for the 3199del6 allele. Carriers of a single I148T allele were also tested for 3199del6.

Individuals homozygous for I148T or compound heterozygous for I148T and a severe CF allele were ascertained from January 1, 1998, to December 1, 2001. They were not selected on the basis of ethnicity. When carrier testing revealed two CF alleles, only those individuals older than 18 years of age were considered apparently healthy adults.

Studies to determine the frequency of 3199del6 in non-CF chromosomes were performed on anonymized DNA from Caucasian individuals. The 95 CF patients with one or no identified CF chromosomes were ascertained from the University of North Carolina and were of various ethnic backgrounds.

CFTR mutation analysis

Genomic DNA was extracted from peripheral blood lymphocytes or buccal cell swabs by Qiagen QIAamp 96 DNA Blood Kit. All samples were tested for 70 to 87 mutations by a pooled allele-specific oligonucleotide (ASO) hybridization method described previously.^{23,24} Nineteen regions of the *CFTR* gene were amplified in two multiplex polymerase chain reactions (PCRs). The amplified PCR products were immobilized on nylon membrane (ICN, Irvine, CA) and hybridized with groups of radiolabeled ASO probes. Individual mutation identification of pool positive samples was made by individual ASO hybridization to normal and mutant alleles.

Sequence analysis

To search for additional sequence changes in individuals who carried the I148T allele, coding exons of the *CFTR* gene were se-

quenced by single PCR amplifications spanning each exon followed by BigDye terminator cycle sequencing. An ABI 310 automated sequencer was used to analyze the cycle sequencing products. The entire *CFTR* coding region and intron-exon boundaries were sequenced in one patient (patient 20).

Intron 8 polythymidine [poly(T)] tract analysis was performed by sequencing or by PCR amplification of a 560-bp region surrounding the polymorphic tract followed by ASO hybridization with probes specific to the 5, 7, or 9T alleles.

Analysis for the 3199del6 sequence change

Analysis of samples for 3199del6 was initially performed by PCR amplification of exon 17a followed by BigDye terminator cycle sequencing. An ABI 310 automated sequencer was used to analyze the cycle sequencing products. To analyze additional samples, a higher throughput denaturing high-performance liquid chromatography (DHPLC) method was developed and validated. Rapid analysis for the 3199del6 mutation was performed by PCR amplification of a 253-bp region flanking exon 17a followed by DHPLC analysis on Varian Prostar Helix. At a mobile phase gradient of 45% B to 68% B and a running temperature of 50°C, the deletion was resolved as an additional peak running approximately 17 seconds ahead of the normal allele. The B mobile phase consisted of 100 mM triethylammonium acetate (TEAA), pH 7, 0.1 mM EDTA, 25% acetonitrile. The A mobile phase consisted of 100 mM TEAA, pH 7, 0.1 mM EDTA.

Statistical analysis

The difference between the frequency of 3199del6 in apparently healthy adult individuals compared with the frequency in those with a known or suspected diagnosis of CF was tested by the Fisher exact test. A *P* value of < 0.05 was considered to indicate statistical significance.

RESULTS

Frequency of I148T in carriers, CF patients, and suspected CF patients

Carrier testing of 42,784 individuals without a family history of CF identified 1,754 individuals who carried one mutation (carrier frequency approximately 1/24). One hundred thirteen of the identified mutations were I148T (6.4%). Diagnostic testing of 4,318 individuals with a clinical diagnosis of CF identified 7,306 mutations. However, only 6 (0.06%) of the affected CF chromosomes were I148T. This represents a 106-fold difference in frequency of I148T between the carrier and affected groups. In a group of 10,231 patients referred for testing due to a suspected diagnosis of CF, 1.1% of the identified CF chromosomes were I148T.

CFTR analysis of apparently healthy individuals and CF patients with the I148T allele

Carrier testing identified seven compound heterozygotes ($\Delta F508$ /I148T) and one I148T homozygote who were apparently unaffected with CF (Table 1). At the time of testing, they

Table 1

3199del6 analysis in apparently healthy adult individuals compound heterozygous or homozygous for I148T

Indiv no.	Indication for testing	Ethnicity	Age (yr)	Mutation 1	Mutation 2	Intron 8 poly(T)	3199del6
12	Carrier testing w/FH	NEC	56	I148T	I148T	9, 9	neg
13	Screening	NEC	40	ΔF508	I148T	9, 9	neg
14	Carrier testing w/FH	Hispanic	32	ΔF508	I148T	9, 9	neg
15	Carrier testing w/FH	NEC	29	ΔF508	I148T	9, 9	neg
16	Carrier testing w/FH	NEC	21	ΔF508	I148T	9, 9	neg
23	Screening	NEC	34	ΔF508	I148T	9, 9	neg
38	Carrier testing w/FH	NEC, SEC	31	ΔF508	I148T	7, 9	neg
40	Carrier testing	Caucasian	30	ΔF508	I148T	9, 9	neg

FH, family history; NEC, Northern European Caucasian; SEC, Southern European Caucasian.

Table 2

3199del6 analysis in patients with a known or suspected diagnosis of CF and compound heterozygous for I148T and a severe CF mutation

Indiv no.	Indication for testing	Ethnicity	Age (yr)	Mutation 1	Mutation 2	Intron 8 poly(T)	3199del6
17	Suspected CF	NEC	4	ΔF508	I148T	9, 9	pos
18	Suspected CF	Caucasian, N. American	1	ΔF508	I148T	9, 9	pos
19	Affected CF	NEC	<1	N1303K	I148T	9, 9	pos
20	Suspected CF	NEC	5	ΔF508	I148T	9, 9	neg ^a
22	Affected CF	NEC	14	ΔF508	I148T	9, 9	pos
39	Pancreatic steatorrhea	NEC	3	ΔF508	I148T	9, 9	pos
41	Suspected CF	Hispanic	10	Q890X	I148T	7, 9	pos
42	Affected CF	NEC	20	ΔF508	I148T	9, 9	pos

NEC, Northern European Caucasian.

^aNo additional mutations were detected.

were 21 to 56 years of age. The presence of the I148T and ΔF508 on the same chromosome was ruled out in two individuals by genotyping of additional family members.

Diagnostic testing of individuals affected with CF, or suspected of having CF, identified eight individuals who were compound heterozygous for a severe mutation (ΔF508, N1303K, or Q890X) and I148T (Table 2). At the time of testing, they were <1 year to 20 years of age. Because of the notably different phenotypes in individuals with similar genotypes, we sequenced regions of the *CFTR* gene from both healthy and affected individuals to determine whether an intragenic change was influencing the phenotype.

Analysis of the intron 8 polythymidine tract determined that there was no significant difference in the poly(T) background of the I148T chromosomes between the healthy and affected groups (Tables 1 and 2). The I148T allele appears to occur most frequently on a 9T chromosomal background. In two individuals (nos. 38 and 41) who carried a 7T and a 9T, it was not possible to determine phase. However, in individual 38 it is most likely that the ΔF508 is on the 9T chromosome and the I148T is on the 7T chromosome.⁷

Analysis of additional *CFTR* exons from healthy and affected patients identified a 6-bp deletion at nucleotide 3199 in some individuals. The deletion is in-frame and results in loss of

an isoleucine and a valine at positions 1023 and 1024 of exon 17a. This exon is part of the 10th transmembrane segment (TM) of the second membrane spanning domain (MSD2). Seven of the eight individuals with a diagnosis of CF, or suspected of having CF, carried the deletion (Table 2). Individual 20, who did not carry the deletion, was 5 years old at the time of testing and was suspected of having CF. Follow-up with the physician indicated that the patient had normal sweat chloride concentrations and good pulmonary function; symptoms of asthma and intermittent respiratory infections were treated with oral antibiotics. Loose stools were resolved with enzyme supplementation. The patient is without *Pseudomonas aeruginosa* infection. Sequencing of all exons did not identify any *CFTR* mutations in addition to I148T and ΔF508. None of the eight apparently healthy individuals (ΔF508/I148T or I148T/I148T) carried the deletion. The frequency of 3199del6 in individuals who are compound heterozygous or homozygous for I148T and not affected with CF versus those who are affected with or suspected of having CF is significantly different ($P = 0.0014$ by Fisher exact test).

Analysis of 3199del6 on other chromosomal backgrounds

Inasmuch as one individual in the healthy group is presumed to carry I148T on a 7T chromosomal background, we

Table 3

3199del6 and poly(T) analysis in individuals who carry one copy of I148T

Intron 8 poly (T)	Indication for testing	no. of individuals tested	3199del6 positive
9, 9	Screening	5	0
	Carrier testing w/FH	1	0
	Echogenic bowel in fetus	1	0
	Suspected CF	1	0
7, 9	Screening	41	1
	Carrier testing w/FH	5	0
	Echogenic bowel in fetus	1	0
	Gamete donor	4	0
	Suspected CF	8	0
	Suspected pancreatitis	3	0
	Not provided	2	1
7, 7	Screening	7	0
	Carrier testing w/FH	1	0
	Gamete donor	3	0
	Suspected CF	1	0
	Suspected pancreatitis	2	0
5, 9	Screening	2	0
	Suspected CF	1	0
5, 7	Carrier testing w/FH	1	0
Total		90	2

FH, family history.

tested 14 individuals who carried one copy of I148T, and who were homozygous for 7T, for the 3199del6 deletion (Table 3). From this analysis, the I148T-7T haplotype does not appear to carry the 3199del6 deletion. Poly(T) analysis of 76 additional individuals who carried one copy of I148T identified 8 who were homozygous for 9T and 64 who were compound heterozygous for 7T and 9T. The deletion was identified in only 2 individuals, both of whom were 7T/9T. Of those individuals whose indication for testing was screening, 1 of 55 I148T carriers also carried the deletion. Since we did not identify any individuals who were 5T/5T, we were unable to determine whether I148T occurs on a 5T background.

To determine whether 3199del6 occurred on CF chromosomes without I148T, we analyzed 95 CF patients with one or no identified *CFTR* mutations for the deletion. None of these patients carried the deletion. We also determined that the deletion did not occur on 386 non-CF control chromosomes.

DISCUSSION

With the start of population screening for *CFTR* mutations,^{20,21} there will be the opportunity to identify mutations and determine their frequencies in large populations that are apparently healthy at the time of testing. Disparity in the fre-

quency of mutations in populations affected with CF and healthy populations suggests that a mutation or variant is not completely penetrant as a simple autosomal recessive allele. This was first appreciated when the frequency of R117H in carriers was observed to be 16-fold higher than the frequency in CF patients.²⁵ In the ethnically diverse US population reported here, the I148T allele is 100-fold more frequent in carriers than in CF patients.

Additional evidence for the variable phenotypic effect of a mutation is the occurrence of the same putative genotype in healthy individuals and CF patients. There are now several published reports of $\Delta F508/R117H$ compound heterozygotes that are apparently healthy.^{22,25,26} The R117H mutation was first identified in 1990²⁷ and has been included in most routine CF genotyping programs, increasing the likelihood that these individuals would have been detected as large numbers of individuals were tested. In contrast, while considered a severe mutation, I148T has not been included in most routine testing. This has likely limited the detection of healthy carriers of I148T/ $\Delta F508$ up to this time. In addition, screening of affected CF populations by allele-specific assays continues to identify I148T in those with CF. Full analysis of these patients would have likely identified the 3199del6 mutation or possibly an additional CF mutation.

The 3199del6 mutation was reported to the CF Consortium Mutation Database in 1998, and it was noted to occur on the same chromosome as I148T.²⁸ Our data corroborate this finding since the CF patients with the deletion and I148T also carried one of three different severe CF alleles. One patient who was suspected of having CF did not carry 3199del6. Further investigation of the clinical phenotype indicated mild respiratory symptoms not exclusive to a diagnosis of CF. As no other pathogenic changes were identified when the entire gene was sequenced, it is possible that this patient does not have CF.

The 3199del6 deletion in exon 17a is in-frame and removes an isoleucine and a valine from the MSD2. There are no reports of the functional effect of 3199del6 on *CFTR* function. However, these two amino acids are conserved in several primates, but not in the mouse.²⁹ Only the valine is conserved in the mouse, with the isoleucine replaced by a leucine.¹⁸ Screening 95 CF patients for 3199del6 did not identify the deletion, suggesting that it does not occur alone as a CF-causing allele. However, this relatively small study warrants additional testing of a larger population. The deletion is apparently a rare allele, inasmuch as it was identified in only 2 of 90 individuals who carried one copy of I148T and was not identified in 386 non-CF chromosomes. It appears most likely that 3199del6 arose on a chromosome that carried the I148T mutation, and thus all chromosomes that have the deletion also have I148T.

With more than 1000 sequence changes identified in the *CFTR* gene, some have been shown to occur on the same chromosome.^{1,30} Prediction of the functional and clinical effect of the single sequence change versus the complex allele will provide a substantial challenge. For example, the complex allele composed of R74W and D1270N is known to produce variable phenotypes. In vitro studies of chloride channel function showed that R74W

has little deleterious effect and could be considered a polymorphism.³¹ D1270N has a more substantial effect on chloride channel function in this model, with the greatest effect observed in the cells containing the complex allele of R74W + D1270N. When in combination with a severe allele on the other chromosome, R74W + D1270N is disease-causing; it was identified in a male with CBAVD, rhinitis, and recurrent respiratory infections.³² However, it was also detected in a 25-year-old woman whose only clinical symptom of CF were two positive sweat chloride tests.³³ In addition, genotyping programs that detect alleles of unknown functional consequence, and unknown population frequency, are at risk for erroneously reporting two changes in *trans* that are in fact a complex allele.

We have now determined that the I148T allele occurs on at least three chromosomal backgrounds (I148T + 7T, I148T + 9T, and I148T + 9T + 3199del6) and that only the complex allele, I148T + 9T + 3199del6, appears to be associated with a classic CF phenotype. Our data do not support the occurrence of the deletion alone; therefore, we cannot assess whether the combination of the missense mutation and the deletion on the same chromosome is pathogenic or whether the entire phenotypic effect is due to the deletion. With the current recommended 25 mutation CF screening panel in place,^{20,21} we recommend that an I148T positive result be followed by reflex testing for the 3199del6 mutation until the data presented here and from other laboratories are reviewed by the ACMG/ACOG Committee. The presence of the complex allele in combination with another severe CF allele confers a CF phenotype, whereas I148T alone, even when a severe allele is on the other chromosome, confers no apparent phenotype. Long-term follow-up studies may be necessary to confidently rule out an association with I148T alone and single-organ or late-onset clinical expression.

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References

- Cystic Fibrosis Mutation Data Base. <http://www.genet.sickkids.on.ca/cftr/>.
- Welsh MJ, Ramsey BW, Accurso F, Cutting GR. Cystic fibrosis. In: Scriver CR, Beaudet AL, Sly WS, Valle D, Childs B, Kinzler KW, Vogelstein B, editors. *Inherited and metabolic basis of disease*. New York: McGraw-Hill, 2001:5121–5188.
- Zielenski J. Genotype and phenotype in cystic fibrosis. *Respiration* 2000;67:117–133.
- Claustres M, Guittard C, Bozon D, Chevalier F, Verlingue C, Ferec C, Girodon E, Cazeneuve C, Beinvenu T, Lalau G, Dumur V, Feldmann D, Bieth E, Blayau M, Clavel C, Creveaux I, Malinge M-C, Monnier N, Malzac P, Mittre H, Chomel J-C, Bonnefont J-P, Iron A, Chery M, Des Georges M. Spectrum of *CFTR* mutations in cystic fibrosis and in congenital absence of the vas deferens in France. *Hum Mutat* 2000;16:143–156.
- Noone PG, Knowles MR. "CFTR-opathies": disease phenotypes associated with cystic fibrosis transmembrane regulator gene mutations. *Respir Res* 2001;2:328–332.
- Noone PG, Zhou Z, Silverman LM, Jowell PS, Knowles MR, Cohn JA. Cystic fibrosis gene mutations and pancreatitis risk: relation to epithelial ion transport and trypsin inhibitor gene mutations. *Gastroenterology* 2001;121:1310–1319.
- Chu C-S, Trapnell BC, Currustin S, Cutting GR, Crystal RG. Genetic basis of variable exon 9 skipping in cystic fibrosis transmembrane conductance regulator mRNA. *Nat Genet* 1993;3:151–156.
- Kiesewetter S, Macek M, Davis C, Currustin SM, Chu C-S, Graham C, Shrimpton AE, Cashman SM, Tsui L-C, Mickle J, Amos J, Highsmith WE, Shuber A, Witt DR, Crystal RG, Cutting GR. A mutation in *CFTR* produces different phenotypes depending on chromosomal background. *Nat Genet* 1993;5:274–278.
- Massie RJH, Poplawski N, Wilcken B, Goldblatt J, Byrnes C, Robertson C. Intron-8 polythymidine sequence in Australasian individuals with CF mutations R117H and R117C. *Eur Respir J* 2001;17:1195–1200.
- Bozon D, Zielenski J, Rininsland F, Tsui L-C. Identification of four new mutations in the cystic fibrosis transmembrane conductance regulator gene: I148T, L1077P, Y1092X, 2183AA>G. *Hum Mutat* 1994;3:330–332.
- Castaldo G, Rippa E, Raia V, Salvatore D, Massa C, de Ritis G, Salvatore F. Clinical features of cystic fibrosis patients with rare genotypes. *J Med Genet* 1996;33:73–76.
- Castaldo G, Rippa E, Sebastio G, Raia V, Ercolini P, de Ritis G, Salvatore D, Salvatore F. Molecular epidemiology of cystic fibrosis mutations and haplotypes in southern Italy evaluated with an improved semiautomated robotic procedure. *J Med Genet* 1996;33:475–479.
- De Braekeleer M, Mari C, Verlingue C, Allard C, Leblanc JP, Simard F, Aubin G, Ferec C. Clinical features of cystic fibrosis patients with rare genotypes in Saguenay Lac-Saint-Jean (Quebec, Canada). *Ann Genet* 1997;40:205–208.
- Kristidis P, Bozon D, Corey M, Markiewicz D, Rommens J, Tsui L-C, Durie P. Genetic determination of exocrine pancreatic function in cystic fibrosis. *Am J Hum Genet* 1992;50:1178–1184.
- Bayleran JK, Yan H, Hopper CA, Simpson EM. Frequencies of cystic fibrosis mutations in the Maine population: high proportion of unknown alleles in individuals of French-Canadian ancestry. *Hum Genet* 1996;98:207–209.
- Chevalier-Porst F, Bonardot AM, Gilly R, Chazalotte JP, Mathieu M, Bozon D. Mutation analysis in 600 French cystic fibrosis patients. *J Med Genet* 1994;31:541–544.
- The Cystic Fibrosis Genetic Analysis Consortium. Population variation of common cystic fibrosis mutations. *Hum Mutat* 1994;4:167–177.
- Tata F, Stanier P, Wicking C, Halford S, Kruyer H, Lench NJ, Scambler PJ, Hansen C, Braman JC, Williamson R, Wainwright BJ. Cloning the mouse homolog of the human cystic fibrosis transmembrane conductance regulator gene. *Genomics* 1991;10:301–307.
- Seibert FS, Jia Y, Mathews CJ, Hanrahan JW, Riordan JR, Loo TW, Clarke DM. Disease-associated mutations in cytoplasmic loops 1 and 2 of cystic fibrosis transmembrane conductance regulator impede processing or opening of the channel. *Biochemistry* 1997;36:11966–11974.
- Grody WW, Cutting GR, Klinger KW, Richards CS, Watson MS, Desnick RJ. Laboratory standards and guidelines for population-based cystic fibrosis carrier screening. *Genet Med* 2001;3:456–461.
- ACOG. Preconception and prenatal carrier screening for CF: clinical and laboratory guidelines. Washington, DC: American College of Obstetricians and Gynecologists.
- Rohlf's EM, Sugarman EA, Heim RA, Allitto BA. Frequency of carriers of two cystic fibrosis mutations in an apparently unaffected adult population. *Genet Med* 2001;3:237.
- Heim RA, Sugarman EA, Allitto BA. Improved detection of cystic fibrosis mutations in the heterogeneous U.S. population using an expanded pan-ethnic mutation panel. *Genet Med* 2001;3:168–176.
- Shuber A, Skoletsky J, Stern R, Handelin B. Efficient 12-mutation testing in the *CFTR* gene: a general model for complex mutation analysis. *Hum Mol Genet* 1993;2:153–158.
- Witt DR, Schaefer C, Hallam P, Wi S, Bulumberg B, Fishbach A, Holtzman J, Kornfeld S, Lee R, Nemzer L, Palmer R. Cystic fibrosis heterozygote screening in 5,161 pregnant women. *Am J Hum Genet* 1996;58:823–835.
- White SM, Lucassen A, Norbury G. Cystic fibrosis: a further case of an asymptomatic compound heterozygote. *Am J Med Genet* 2001;103:342–343.
- Dean M, White MB, Amos J, Gerrard B, Stewart C, Khaw K-T, Leppert M. Multiple mutations in highly conserved residues are found in mildly affected cystic fibrosis patients. *Cell* 1990;61:863–870.
- Bozon D. CF Consortium NL 70, March 19, 1998.
- Chen J-M, Cutler C, Jacques C, Boeuf G, Denamur E, Lecointre G, Mercier B, Cramb G, Ferec C. A combined analysis of the cystic fibrosis transmembrane conductance regulator: implications for structure and disease models. *Mol Biol Evol* 2001;18:1771–1788.
- Savov A, Angelicheva D, Balassopoulou, Jordanova A, Noussia-Arvanitakis S, Kalydjieva L. Double mutant alleles: are they rare? *Hum Mol Genet* 1995;4:1169–1171.
- Fanen P, Clain J, Labarthe R, Hulin P, Giordon E, Pagesy P, Goossens M, Edelman A. Structure-function analysis of a double-mutant cystic fibrosis transmembrane conductance regulator protein occurring in disorders related to cystic fibrosis. *FEBS Lett* 1999;452:371–374.
- Casals T, Bassas L, Ruiz-Romero J, Chillón M, Gimenez J, Ramos MD, Tapia G, Narvaez H, Nunes V, Estivill X. Extensive analysis of 40 infertile patients with congenital absence of the vas deferens: in 50% of cases only one *CFTR* allele could be detected. *Hum Genet* 1995;95:205–211.
- Verlingue C, David A, Audrezet MP, Le Roux MG, Mercier B, Moisan JP, Ferec C. Asymptomatic carrier of two *CFTR* mutations: consequences for prenatal diagnosis? *Prenat Diag* 1993;13:1143–1148.