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Identification of the M1101K Mutation in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) Gene and Complete Detection of Cystic Fibrosis Mutations in the Hutterite Population

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Summary

The Hutterite population is a genetic isolate with an increased incidence of cystic fibrosis (CF). Previously we identified three CF haplotypes defined by polymorphisms flanking the CF transmembrane conductance regulator (CFTR) gene. ΔF508 was present on one of the haplotypes in only 35% of CF chromosomes. We hypothesized that the other two CF haplotypes, one of which was the most common and the other of which is rare, each harbored different non-ΔF508 mutations. Single-strand conformation polymorphism analysis detected a missense mutation, M1101K, in both chromosomes of a Hutterite patient carrying the two non-ΔF508 haplotypes. M1101K appears to have originated on an uncommon CFTR allele and to be infrequent outside the Hutterite population. The presence of M1101K on two haplotypes is likely the result of a CFTR intragenic recombination which occurred since the founding, 10–12 generations ago, of the Hutterite population. The crossover was located between exons 14a and 17b, an interval of approximately 15 kbp. ΔF508 and M1101K accounted for all of the CF mutations in patients from 16 CF families representing the three subdivisions of the Hutterite population.

Introduction

ΔF508 is the most common cystic fibrosis (CF) mutation in Caucasian populations (Kerem et al. 1989; Cystic Fibrosis Genetic Analysis Consortium 1990; European Working Group on CF Genetics 1990). There is a gradient in the proportion of ΔF508, which increases from about 50% in southern Europe to more than 75% in northern Europe. Over 200 CF transmembrane conductance regulator (CFTR) mutations have been reported by members to the Cystic Fibrosis Genetic Analysis Consortium (Tsui 1992). With the exception of a few other mutations with proportions greater than 5% in one or more populations (Liechti-Gallati et al. 1991; Nunes et al. 1991; Shrimpton et al. 1991; Abeliovich et al. 1992; Cuppens et al. 1992; Rozen et al. 1992; Shoshani et al. 1992), most non-ΔF508 mutations are rare. A subset of non-ΔF508 mutations accounts for a total of about 10% of CF mutations in most Caucasian populations (Cutting et al. 1992). In certain well-defined populations, more than 90% of CF mutations can be detected by testing for a small number of CFTR mutations (Abeliovich et al. 1992; Férec et al. 1992; Rozen et al. 1992; Shoshani et al. 1992). Screening the entire coding region and sequences flanking the 27 exons of CFTR by using denaturing gradient gel electrophoresis resulted in the identification of (*a*) 28 different mutations which accounted for 88% of the mutations in French CF patients (Fanen et al. 1992) and (*b*) 19 different mutations which accounted for 98% of the

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mutations in Celtic CF families from Brittany (Férec et al. 1992). Even direct sequencing may not identify all mutations in heterogeneous populations, since some CF mutations may be located outside the regions of the CFTR gene which are commonly targeted for screening: direct sequencing of the entire CFTR coding region and introns in the vicinity of splice junctions did not identify the mutation in 7 of 96 CF chromosomes in Canadian CF families (J. Zielenski and L.-C. Tsui, unpublished observation).

The Hutterites or Hutterian Brethren are German-speaking communal farmers who live on the Great Plains of North America (Hostetler 1985). There are about 30,000 Hutterites, and the population is subdivided into three endogamous groups: the Dariusleut, Lehrerleut, and Schmiedeleut. The Hutterite population is a genetic isolate with an average inbreeding coefficient of about .05 for a recent cohort (Thompson and Morgan 1989). Genealogical information for as many as 12 ancestral generations is available for most of the contemporary Hutterites (T. M. Fujiwara and K. Morgan, unpublished observation). Founder effect is responsible for an increased incidence of CF in the inbred Hutterite population (Fujiwara et al. 1989). We estimated that the prevalence of CF carriers in the Hutterite population was 11%, on the basis of CF incidence in the Hutterite population of Alberta, Canada. Furthermore, on the basis of haplotype analysis, it was anticipated that there are as few as two other CF mutations in addition to ΔF508 in this population (Fujiwara et al. 1989; Klinger et al. 1990). Here we report the identification of the most common CF mutation in the Hutterite population, M1101K, a missense mutation in exon 17b. We also infer that a CFTR intragenic crossover occurred which resulted in two haplotypes harboring the M1101K mutation.

Patients, Material, and Methods

Patients Studied

DNA from a patient who carried two different CF haplotypes harboring non-ΔF508 mutations was analyzed. Lymphoblast cultures from this family are available from the National Institute of General Medical Sciences' Human Genetic Mutant Cell Repository: the family number is 1080, and the patient's lymphoblast culture is GM07857. A new CF mutation, M1101K, was identified by single-strand conformation polymorphism (SSCP) analysis. DNA samples from 16 Hutterite CF families were analyzed. Samples were obtained by informed consent. Ten of the families were described elsewhere (Fujiwara et al. 1989; Klinger et al. 1990). DNA samples of patients from six additional Alberta Hutterite families were provided by L. S. Dimnik and A. T. Garber (Molecular Diagnostic Laboratory, Alberta Hereditary Diseases Program, Southern Region, Alberta Children's Hospital, Calgary).

SSCP Analysis and DNA Sequencing

For SSCP analysis (Orita et al. 1989), a 263-bp fragment corresponding to the 3′ portion of exon 17b was amplified by PCR (Saiki et al. 1988). Genomic DNA preparations were amplified and labeled using the primers 17Bi-5s (5′-TATGGACACTTCGTGCCTTC-3′) and 17Bi-3 (5′-ATAACCTATAGAATGCAGCA-3′). The 25-μl reactions contained 100 ng of genomic DNA; 10 mM Tris-HCl, pH 8.3; 50 mM KCl; 1.75 mM $MgCl_2$; 0.001% gelatin; 0.2 mM each dATP, dGTP, and dTTP; 0.08 mM dCTP; 10 pmol of each primer; 2.5 μCi of (α-^{32}P)-dCTP (3,000 Ci/mmol; Amersham); and 1 unit of *Taq* polymerase. The amplification was carried out in the Perkin Elmer Cetus GeneAmp PCR System 9600 for 30 cycles, each consisting of denaturation at 94°C for 20 s, annealing at 60°C for 20 s, and elongation at 72°C for 30 s, followed by one cycle of 12 s at 94°C, 20 s at 60°C, and 7 min at 72°C. For electrophoresis, 1.5 μl of PCR reaction product was mixed with 9 μl of 10 mM NaOH, 0.05% bromophenol blue, and 0.05% xylene cyanol in 95% formamide and was denatured by being heated for 5 min at 95°C before being loaded onto a 6% polyacrylamide gel containing 10% glycerol. The gel was run at 20 mA overnight (16–20 h) at room temperature, was dried, and was autoradiographed. Direct sequencing of PCR-amplified mutant and normal DNA was performed according to a method described elsewhere (Zielenski et al. 1991*a*).

Mutation Detection

Detection of the M1101K mutation was performed by dot blot analysis. DNA was amplified by PCR using the pair of primers, 17Bi-5 and 17Bi-3, to give a 463-bp product (Zielenski et al. 1991*c*). The normal sequence was detected by the 16-mer allele-specific oligonucleotide 5′-GTTCCAAA*T*GAGAATA-3′, and the mutant sequence was detected by 5′-GTTCCAAA*A*GAGAATA-3′. Nylon blotting membranes (Zeta-Probe; Bio-Rad) were hybridized in 5 × SSPE (20 × SSPE = 3 M NaCl, 20 mM NaH_2PO_4, and 20 mM EDTA), 0.5% SDS, and 5 × Denhardt's reagent at 37°C for 1 h and were washed in 2 × SSPE and 0.1% SDS at 42°C for 10

min. The film was exposed with an intensifying screen to the membrane at −80°C for 1 h. Testing for ΔF508 was done according to the protocol of Rommens et al. (1990).

Haplotype Analysis

Haplotype analysis of DNA polymorphisms flanking the CF locus was previously reported for 10 Hutterite CF families (Fujiwara et al. 1989; Klinger et al. 1990). Ten additional polymorphisms, all but one of which were located in the CFTR gene, were analyzed by PCR in two Hutterite families, HT01 and HT02, to further characterize the three CF haplotypes *a, b,* and *c;* these polymorphisms are J44/*Xba*I, which is located approximately 7 kb 5′ to CFTR exon 1 (Kerem et al. 1989; Rommens et al. 1989; Dörk et al. 1992); a 4-bp repeat in intron 6a, $(GATT)_n$ (Chehab et al. 1991; Gasparini et al. 1991; Klinger et al. 1991); a dinucleotide repeat in intron 8, $(CA)_n$ (Morral et al. 1991); a nucleotide substitution in intron 9, 1525–61 (A or G) (Dörk et al. 1992); two nucleotide substitutions in exon 10, 1540 (A or G) and 1716 (G or A) (Kerem et al. 1990); a nucleotide substitution in intron 12, 1898+152 (T or A) (Chillón et al. 1991); a nucleotide substitution in exon 14a, 2694 (T or G) (Kerem et al. 1990); a dinucleotide repeat in intron 17b, $(TA)_n$ (Zielenski et al. 1991*b*); and a nucleotide substitution in intron 18, 3601–65 (C or A) (Dörk et al. 1991).

Results

The DNA from a Hutterite CF patient (family HT02) was included in a panel of DNA samples obtained from a large group of CF patients who had at least one non-ΔF508 mutation. The panel was screened for CF mutations by SSCP analysis of the following 15 exons and the corresponding flanking intron sequences: 2–5, 7, 9–13, 17b, and 19–22. The total region tested by SSCP analysis corresponds to 64% of the CFTR coding sequence. (The experimental conditions for SSCP analysis of the exons other than 17b will be reported elsewhere.) A missense mutation, M1101K, in exon 17b was detected by SSCP analysis of a 263-bp segment of DNA amplified by PCR using the primers 17Bi-5s and 17Bi-3. Sequencing analysis identified the mutation as a T-to-A transversion at position 3434, leading to a predicted change of methionine (codon 1101, ATG) to lysine (codon AAG) (fig. 1). The mutation was found on both chromosomes of the Hutterite CF patient.

The three CF children in family HT02 were examined by R.H.S., and sweat tests were done at the time of blood sampling (Fujiwara et al. 1989) (table 1). For comparison of the patients' sweat osmolality values, mean osmolality of 46 normal individuals was 115 mmol/kg (SD = 28); the parents' osmolality values were 106 and 107 mmol/kg. For all three children there was clinical evidence of fat maldigestion, which responded to supplemental pancreatic enzymes. One of the children had a right-upper-lobe lobectomy. There was clinical variability in that the oldest child appeared to have the best clinical condition. The sisters are presently ages 22 and 19 years. Their brother died at age 17 years.

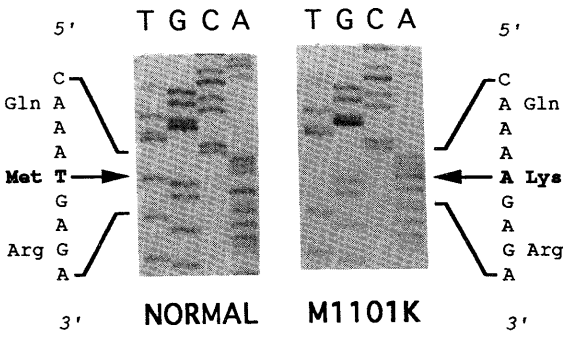

Figure 1 Sequence analysis of the mutation, M1101K, detected by SSCP analysis in a patient from Hutterite family HT02. The arrows indicate the nucleotide change, T to A, which results in a substitution of lysine for methionine at CFTR residue 1101.

All CF mutations were identified in patients from the 16 Hutterite CF families: 22/32 (69%) CF chromosomes carried M1101K, and the remaining 10/32 (31%) carried ΔF508. The three CF haplotypes, previously defined by polymorphisms flanking the CFTR locus, in the Hutterite families were designated haplotypes "*a*," "*b*," and "*c*" (Fujiwara et al. 1989) (fig. 2). Haplotype *a* harbors ΔF508 and carries $(GATT)_6$ and $(TA)_{32}$ repeat alleles. All CF chromosomes which harbor ΔF508 carry $(GATT)_6$ (Chehab et al. 1991; Dörk et al. 1992), and $(TA)_{32}$ was present in 22/61 (36%) of ΔF508 alleles (Zielenski et al. 1991*b*). Haplotypes *b* and *c* both harbor M1101K and carry $(TA)_{29}$. M1101K and $(TA)_{29}$ are only 265 bp apart (Zielenski et al. 1991*c*).

M1101K was present on two different haplotypes, *b* and *c,* in the Hutterite population, most likely as a result of intragenic crossing-over. It was possible to define the region of the proximal crossover by using 10 polymorphisms in the 5′ flanking region of CFTR and within the gene (fig. 2). Comparison of haplotypes *b* and *c* (fig. 3) locates the crossover between nucleotide

Table 1

CF in Siblings of Family HT02

Gender of Patient	Age at Initial Exam	Meconium Ileus	Nasal Polyps	Clubbing of Digits	Sweat Osmolality (mmol/kg)
Female	13 years 11 mo	No	Yes	No	275
Female	11 years 3 mo	No	No	Yes	283
Male	10 years	No	Yes	Yes	245

2694 in exon 14a and nucleotide 3434 in exon 17b of the CFTR coding sequence, which corresponds to a distance of approximately 15 kb in the CFTR gene (Rommens et al. 1989) (fig. 2).

Discussion

We propose that M1101K is a CF mutation, because the transversion of T to A is predicted to result in the substitution of a basic for a nonpolar amino acid. Three Hutterite siblings who were homozygous for M1101K had pancreatic insufficiency. Another mutation, M1101R, which is a transversion of T to G at the same nucleotide position and should also result in a basic amino acid substitution, was identified by other investigators in a Turkish CF patient who was ΔF508/M1101R and had pancreatic insufficiency (W. Lissens, M. Bonduelle, I. Liebaers, C. Férec, I. Quere, B. Mercier, and M. P. Audrezet, personal communication). In addition, the methionine at codon 1101 is conserved in human (Riordan et al. 1989), bovine (Diamond et al. 1991), and murine (Tata et al. 1991; Yorifuji et al. 1991) CFTRs.

M1101K is the most common mutation in the Hutterite CF patients. It accounted for 69% of CF chromosomes, while ΔF508 accounted for the remaining 31%. The CFTR allele harboring M1101K carries the infrequent IVS17b $(TA)_{29}$ repeat allele. In Canadian CF families, only 3% of normal alleles and no CF alleles carried $(TA)_{29}$ (Zielenski et al. 1991*b*). M1101K appears to be rare outside the Hutterite population, because the mutation has not been found after screening 328 other CF

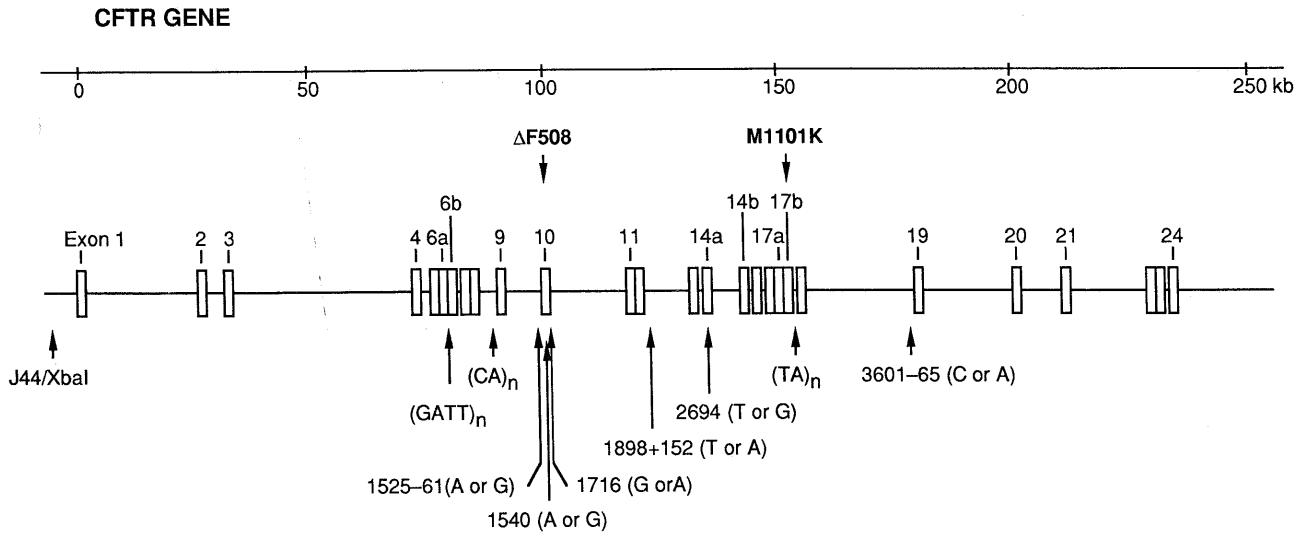

Figure 2 Locations of the ΔF508 and M1101K mutations and the polymorphisms used in the haplotype analysis of two Hutterite families (for details, see text and fig. 3). The schematic diagram of the CFTR gene exon/intron organization was adapted from Zielenski et al. (1991*c*).

	a	b	c	H	K	M	N
metD/TaqI	1	1	1	1	2	1	1
metH/TaqI	1	2	1	1	1	1	2
XV-2c/TaqI	1	2	2	2	1	2	2
KM.19/PstI	2	1	2	1	2	2	2
J44/XbaI	1	2	1	2	1	1	1
$(GATT)_n$	6	7	7	7	7	7	7
$(CA)_n$	17	17	16	16	16	15	16
1525–61 (A or G)	1	2	2	2	2	2	2
1540 (A or G)	1	2	1	2	1	1	1
ΔF508	ΔF508	N	N	N	N	N	N
1716 (G or A)	2	2	1	2	2	2	2
1898+152 (T or A)	1	1	2	1	2	2	2
2694 (T or G)	1	1	2	1	2	2	2
M1101K	N	M1101K	M1101K	N	N	N	N
$(TA)_n$	32	29	29	30	7	7	7
3601–65 (C or A)	1	1	1	1	2	2	2
J3.11/MspI	1	2	2	1	1	1	1

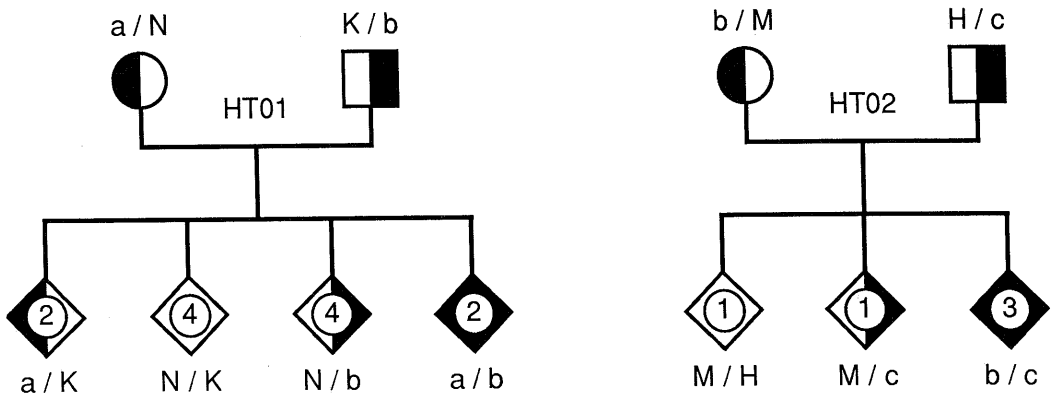

Figure 3 CF (*a*, *b*, and *c*) and normal (*H*, *K*, *M*, and *N*) haplotypes in two Hutterite families. DNA samples from both parents and at least one offspring of each CFTR genotype were analyzed. The numbers of offspring studied are indicated by numbers enclosed in circles. The polymorphisms and CF mutations are given in proximal-to-distal order in chromosome region 7q31 and span approximately 1,490 kb (Rommens et al. 1989). KM.19 (also known as JG2E1) is about 130 kb 5′ to the CFTR gene. The locations of CFTR mutations and polymorphisms are given in fig. 2 and the text. J3.11 is located more than 500 kb 3′ to the CFTR gene.

chromosomes (140 harboring ΔF508, 52 with identified non-ΔF508 mutations, and 136 with unidentified mutations). All 92 normal chromosomes tested were also negative for the mutation.

M1101K is present on two CF haplotypes, *b* (most common) and *c* (rare), in the Hutterite population as a result of crossing-over. The crossover occurred in an interval of about 15 kb between exons 14a and 17b. Haplotype *c* has so far been found in only one Schmiedeleut family (fig. 3). Additional CF and normal chromosomes in CF families from the three Hutterite subdivisions need to be haplotyped to provide information about the time of the crossover which generated haplotype *c*. Since all of the CF mutations have been detected in patients from 16 families who are from all three subdivisions of the Hutterite population, it is likely that all CF mutations have been identified in this population. The introduction of other CF mutations by recent im-

migrants would have a small probability when the carrier prevalence of non-ΔF508 mutations in the general population and the low rate of migration into this genetic isolate are taken into consideration. We will attempt to identify the likely original carriers of the ΔF508 and M1101K alleles in the Hutterite population by genealogical analyses. It may prove informative to test for the M1101K mutation in CF patients in Austria and Germany who have unidentified CF mutations. A large proportion of the Hutterites descend from a group of founder families who came from Carinthia in southern Austria (Hostetler 1985).

By testing for only two CFTR mutations, ΔF508 and M1101K, it is feasible to obtain reliable estimates of carrier prevalence in the Hutterite population. For example, if the carrier prevalence was 11% (Fujiwara et al. 1989), the 95% confidence interval for a random sample of 100 Hutterite adults would be approximately 6–19 carriers. Furthermore, accurate carrier testing and genetic counseling can be offered to Hutterite adults who may want this information (Miller and Schwartz 1992). DNA analysis can aid early diagnosis of CF in Hutterite infants. This would facilitate early treatment of the disease, which, it is hoped, would contribute to improved prognosis for the patients.

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References

Abeliovich D, Lavon IP, Lerer I, Cohen T, Springer C, Avital A, Cutting GR (1992) Screening for five mutations detects 97% of cystic fibrosis (CF) chromosomes and predicts a carrier frequency of 1:29 in the Jewish Ashkenazi population. Am J Hum Genet 51:951–956

Chehab FF, Johnson J, Louie E, Goossens M, Kawasaki E, Erlich H (1991) A dimorphic 4-bp repeat in the cystic fibrosis gene is in absolute linkage disequilibrium with the ΔF508 mutation: implications for prenatal diagnosis and mutation origin. Am J Hum Genet 48:223–226

Chillón M, Nunes V, Estivill X (1991) SSCP-polymorphism in intron 12 of the CFTR gene recognized by BclI. Nucleic Acids Res 19:6343

Cuppens H, Buyse I, Baens M, Marynen P, Cassiman J-J (1992) Simultaneous screening for 11 mutations in the cystic fibrosis transmembrane conductance regulator gene by multiplex amplification and reverse dot-blot. Mol Cell Probes 6:33–39

Cutting GR, Curristin SM, Nash E, Rosenstein BJ, Lerer I, Abeliovich D, Hill A, et al (1992) Analysis of four diverse population groups indicates that a subset of cystic fibrosis mutations occur in common among Caucasians. Am J Hum Genet 50:1185–1194

Cystic Fibrosis Genetic Analysis Consortium (1990) Worldwide survey of the ΔF508 mutation—report from the Cystic Fibrosis Genetic Analysis Consortium. Am J Hum Genet 47:354–359

Diamond G, Scanlin TF, Zasloff MA, Bevins CL (1991) A cross-species analysis of the cystic fibrosis transmembrane conductance regulator. J Biol Chem 266:22761–22769

Dörk T, Neumann T, Wulbrand U, Wulf B, Kälin N, Maaß G, Krawczak M, et al (1992) Intra- and extragenic marker haplotypes of CFTR mutations in cystic fibrosis families. Hum Genet 88:417–425

Dörk T, Wulbrand U, Tümmler B (1991) A HinfI polymorphism in the cystic fibrosis gene CFTR. Nucleic Acids Res 19:2517

European Working Group on CF Genetics (1990) Gradient of distribution in Europe of the major CF mutation and of its associated haplotypes. Hum Genet 85:436–441

Fanen P, Ghanem N, Vidaud M, Besmond C, Martin J, Costes B, Plassa F, et al (1992) Molecular characterization of cystic fibrosis: 16 novel mutations identified by analysis of the whole cystic fibrosis conductance transmembrane regulator (CFTR) coding regions and splice site junctions. Genomics 13:770–776

Férec C, Audrezet MP, Mercier B, Guillermit H, Moullier P, Quere I, Verlingue C (1992) Detection of over 98% cystic fibrosis mutations in a Celtic population. Nature Genet 1:188–191

Fujiwara TM, Morgan K, Schwartz RH, Doherty RA, Miller SR, Klinger K, Stanislovitis P, et al (1989) Genealogical analysis of cystic fibrosis families and chromosome 7q RFLP haplotypes in the Hutterite brethren. Am J Hum Genet 44:327–337

Gasparini P, Dognini M, Bonizzato A, Pignatti PF, Morral N, Estivill X (1991) A tetranucleotide repeat polymorphism in the cystic fibrosis gene. Hum Genet 86:625

Hostetler JA (1985) History and relevance of the Hutterite

population for genetic studies. Am J Med Genet 22:453–462

Kerem B, Rommens JM, Buchanan JA, Markiewicz D, Cox TK, Chakravarti A, Buchwald M, et al (1989) Identification of the cystic fibrosis gene: genetic analysis. Science 245:1073–1080

Kerem B, Zielenski J, Markiewicz D, Bozon D, Gazit E, Yahav J, Kennedy D, et al (1990) Identification of mutations in regions corresponding to the two putative nucleotide (ATP)-binding folds of the cystic fibrosis gene. Proc Natl Acad Sci USA 87:8447–8451

Klinger K, Horn GT, Stanislovitis P, Schwartz RH, Fujiwara TM, Morgan K (1990) Cystic fibrosis mutations in the Hutterite brethren. Am J Hum Genet 46:983–987

Klinger KW, Stanislovitis P, Merrill J, Horn GT (1991) Molecular and genetic analysis at the CF locus. Adv Exp Med Biol 290:39–43

Liechti-Gallati S, Malik N, Alkan M, Maechler M, Morris M, Thonney F, Sennhauser F, et al (1991) Association between haplotypes and specific mutations in Swiss cystic fibrosis families. Pediatr Res 30:304–308

Miller SR, Schwartz RH (1992) Attitudes toward genetic testing of Amish, Mennonite, and Hutterite families with cystic fibrosis. Am J Public Health 82:236–242

Morral N, Nunes V, Casals T, Estivill X (1991) CA/GT microsatellite alleles within the cystic fibrosis transmembrane conductance regulator (CFTR) gene are not generated by unequal crossingover. Genomics 10:692–698

Nunes V, Gasparini P, Novelli G, Gaona A, Bonizzato A, Sangiuolo F, Balassopoulou A, et al (1991) Analysis of 14 cystic fibrosis mutations in five South European populations. Hum Genet 87:737–738

Orita M, Suzuki Y, Sekiya T, Hayashi K (1989) Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. Genomics 5:874–879

Riordan JR, Rommens JM, Kerem B, Alon N, Rozmahel R, Grzelczak Z, Zielenski J, et al (1989) Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. Science 245:1066–1073

Rommens JM, Iannuzzi MC, Kerem B, Drumm ML, Melmer G, Dean M, Rozmahel R, et al (1989) Identification of the cystic fibrosis gene: chromosome walking and jumping. Science 245:1059–1065

Rommens J, Kerem B, Greer W, Chang P, Tsui L-C, Ray P (1990) Rapid nonradioactive detection of the major cystic fibrosis mutation. Am J Hum Genet 46:395–396

Rozen R, De Braekeleer M, Daigneault J, Ferreira-Rajabi L, Gerdes M, Lamoureux L, Aubin G, et al (1992) Cystic fibrosis mutations in French Canadians: three CFTR mutations are relatively frequent in a Quebec population with an elevated incidence of cystic fibrosis. Am J Med Genet 42:360–364

Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, et al (1988) Primer-directed enzymatic amplification of DNA with thermostable DNA polymerase. Science 239:487–491

Shoshani T, Augarten A, Gazit E, Bashan N, Yahav Y, Rivlin Y, Tal A, et al (1992) Association of a nonsense mutation (W1282X), the most common mutation in the Ashkenazi Jewish cystic fibrosis patients in Israel, with presentation of severe disease. Am J Hum Genet 50:222–228

Shrimpton AE, McIntosh I, Brock DJH (1991) The incidence of different cystic fibrosis mutations in the Scottish population: effects on prenatal diagnosis and genetic counselling. J Med Genet 28:317–321

Tata F, Stanier P, Wicking C, Halford S, Kruyer H, Lench NJ, Scambler PJ, et al (1991) Cloning the mouse homolog of the human cystic fibrosis transmembrane conductance regulator gene. Genomics 10:301–307

Thompson EA, Morgan K (1989) Recursive descent probabilities for rare recessive lethals. Ann Hum Genet 53:357–374

Tsui L-C (1992) The spectrum of cystic fibrosis mutations. Trends Genet 8:392–398

Yorifuji T, Lemna WK, Ballard CF, Rosenbloom CL, Rozmahel R, Plavsic N, Tsui L-C, et al (1991) Molecular cloning and sequence analysis of the murine cDNA for the cystic fibrosis transmembrane conductance regulator. Genomics 10:547–550

Zielenski J, Bozon D, Kerem B, Markiewicz D, Durie P, Rommens JM, Tsui L-C (1991*a*) Identification of mutations in exons 1 through 8 of the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Genomics 10:229–235

Zielenski J, Markiewicz D, Rininsland F, Rommens J, Tsui L-C (1991*b*) A cluster of highly polymorphic dinucleotide repeats in intron 17b of the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Am J Hum Genet 49:1256–1262

Zielenski J, Rozmahel R, Bozon D, Kerem B, Grzelczak Z, Riordan JR, Rommens J, et al (1991*c*) Genomic DNA sequence of the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Genomics 10:214–228