Association of less common cystic fibrosis mutations with a mild phenotype

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Abstract

A majority of cystic fibrosis (CF) genes (70 to 75%) share a single mutation, but the remaining 25 to 30% of defects are accounted for by more than 20 different mutations. One of the less frequent mutations, G551D, has been identified in the CF genes of two sibs and one unrelated adult patient. The adult patient also has a second rare mutation, Δ I507. All three subjects exhibit a less severe phenotype than that normally associated with CF. This supports a hypothesis that the common mutation (Δ F508) is responsible for the severe form of the disorder, and the minority of patients with a milder form tend to have mutations at other sites in the CF gene.

A deletion of three base pairs in exon 10 of the gene¹ has been shown to be the primary genetic mutation in 68% of chromosomes carrying a defective cystic fibrosis (CF) gene in North America.² This mutation is likely to represent more than 75% of CF mutations in the British population.^{3,4} The deleted triplet (Δ F508) results in the removal of a phenylalanine residue at amino acid position 508, which resides in the putative ATP binding region of the 1480 amino acid CF gene product, the cystic fibrosis transmembrane conductance regulator (CFTR).¹ It was estimated from linkage disequilibrium and haplotype data that a small number of further mutations must exist to account for the remaining 25 to 30% of defective CF loci in Britain and North America.^{2,5} However, a further 20 mutations have currently been found which together make up no more than a few

percent of the remaining defective chromosomes.⁶ One of the less common mutations (G551D), initially described by Cutting *et al*,^{6a} results in the creation of an *Mbo*I recognition site at codon 551 in the CFTR gene. A further mutation, Δ I507, first described by Schwarz *et al*,^{6b} is the result of a three base pair deletion adjacent to the Δ F508 site at codon 507.

A minority (approximately 15%) of persons affected with cystic fibrosis exhibit a milder phenotype than that usually associated with the disorder. These cases often survive into adulthood, the females reproduce, and are pancreatic sufficient.⁷ Many of the severely affected patients, being pancreatic insufficient and approximately 10% having a history of meconium ileus, have been found to be homozygous for the Δ F508 mutation.^{2,3} Those with an apparently less severe form of the disease are predominantly compound heterozygotes at the CF locus. They either have a single mutation at the Δ F508 position and are, therefore, presumed to carry one of the other mutations on the other chromosomes, or do not have the Δ F508 deletion at all and therefore probably carry two of the rarer mutations.

In this study we describe, first, a family with two children who have very mild CF-like symptoms in which the G551D mutation is segregating and, secondly, an unrelated adult CF female who is a compound heterozygote for G551D and Δ I507.

Materials and methods

DNA was extracted by standard methods⁸ from EDTA blood samples collected from the parents and three children of a family in which two of the children had very mild CF-like symptoms, and from a 24 year old female CF patient.

The adult patient, diagnosed as having CF in childhood, had recently married. She was pancreatic sufficient and only attended the clinic intermittently. The two sibs with a mild CF phenotype also had normal exocrine pancreatic function. II.1 (fig 1) is 14 years old. He had severe bronchopneumonia at the age of 4 years and his respiratory tract has had recurrent *Staphylococcus aureus* and *Haemophilus influenzae* infections. Sweat chloride values of a test carried out during a recent admission to hospital were

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Figure 1 Detection of G551D mutation. PAGE of PCR amplified DNA samples using primers which flank the G551D mutant site from members of a mild CF family and a mild CF adult patient. The 420 bp amplified fragment is cut into two fragments of 243 bp and 177 bp by *MboI* when the mutation is present, as shown in II-1, I-1, II-2, and the adult patient.

88 and 53 mmol/l. He has now recovered and is leading a normal, active life. His sister (II-2, fig 1) has suffered from recurrent respiratory problems since the age of 8 months, including *S aureus* and *H influenzae* infections. She has a normal nasal potential difference measurement but a recent sweat chloride test showed borderline values of 67, 67, 44, and 60 mmol/l.

The polymerase chain reaction (PCR)⁹ was used to amplify the genomic DNA flanking the $\Delta F508/\Delta I507$ sites and the G551D site. Reaction mixes contained 0.5 μ g of template DNA, 1 unit *Taq* polymerase (Cetus), each dNTP at 0.2 mmol/l, and 25 pmol of each primer in a total volume of 50 μ l. The initial amplification cycle consisted of 2.5 minutes denaturation at 94°C, 0.5 minutes annealing at 50°C, and one minute extension at 72°C, followed by 34 cycles of 0.5 minutes at 91°C, 0.5 minutes at 50°C, and 0.5 minutes at 72°C, and then a final extension of five minutes at 72°C. Primer sequences are shown in table 1.

The PCR products were analysed for the presence of the $\Delta F508$ deletion or its normal homologue on duplicate dot blots (Gene screen plus, Dupont) by

hybridisation with specific oligonucleotides whose sequences are shown in table 1, or by polyacrylamide gel electrophoresis (PAGE).^{10 11} Hybridisation of dot blots was carried out at 50°C in 4×SSC, 5× Denhardt's solution, and 10% dextran sulphate for four hours. Filters were washed for 15 minutes each at 48°C in 1×SSC, 0.1% SDS, 0.1% sodium pyrophosphate, and then in 0.1×SSC, 0.1% SDS, 0.1% sodium pyrophosphate.

The $\Delta I507$ mutation was detected by PAGE. Samples for electrophoresis were prepared in 5% sucrose, 0.06% orange G. Polyacrylamide gels were prepared from 10% Acrylogel 5 (BDH) in 1×TBE and electrophoresis was at 75V for 15 hours. Gels were analysed by visualisation under UV light following ethidium bromide staining. The presence of $\Delta F508$ and $\Delta I507$ was identified by comparison with known controls.¹¹ The G551 base substitution was identified after digestion of 10 μ l of the PCR amplified DNA with two units of *MboI* (using the manufacturer's recommended procedure) and electrophoresis on Hydrolink-dsDNA gels (AT Biochem) at 50V for 15 hours.

Results

The presence or absence of the mutations at the $\Delta F508$, $\Delta I507$, and G551D positions of the CF gene in the six amplified DNA samples are shown in table 2. The $\Delta F508$ deletion was not detected in any of those tested as shown by dot blot hybridisation (fig 2) and PAGE (fig 3).

Table 2 Results of the mutations detected in the CF genes of the six subjects tested. The family pedigree is shown in fig 1.

Source of DNA	Genotype at mutant site			
	$\Delta F508$	$\Delta I507$	G551D	CF genotype
Father I-1	N/N	N/N	N/X	N/G551D
Mother I-2	N/N	N/N	N/N	N/N*
Son II-1	N/N	N/N	N/X	N/G551D
Daughter II-2	N/N	N/N	N/X	N/G551D
Daughter II-3	N/N	N/N	N/N	N/N*
Unrelated adult patient	N/N	N/X	N/X	$\Delta I507$ /G551D

N=presence of a non-mutant sequence and X=the presence of a mutant sequence.

*Indicates that an unidentified CF mutation may be present in these subjects.

Table 1 Sequences of the forward (F) and reverse (R) primers used in the PCR amplification of the mutant sites, $\Delta F508$, $\Delta I507$, and G551D, and of the hybridisation probes used specifically to detect the $\Delta F508$ deletion and its normal homologue.

Oligonucleotide function	Mutant site	Sequence
PCR primer	$\Delta F508/\Delta I507$	F: 5'GTTTCTCTGGATTATGCCTGGCAC R: 5'GTTGGCATGCTTTGATGACGCTTC
PCR primer	G551D	F: 5'TTCAGCAATGTTGTTTGGACCAAC R: 5'CACAGATTCTGAGTAACCATAATC
Dot blot hybridisation probe	$\Delta F508$	Normal allele: 5'CACCAAAGATGATATTTTC Mutant allele: AACACCAATGATATTTTCTT

Figure 2 Detection of $\Delta F508$ mutation. The normal oligonucleotide probe (upper row) and $\Delta F508$ allele specific oligonucleotide probe (lower row) were hybridised to duplicate dot blots of PCR amplified DNA from members of a mild CF family (lanes 1 to 5) and a mild CF adult patient (lane 6). Control $\Delta F508/\Delta F508$, lane 7. Control N/N, lane 8. Control $\Delta F508/N$, lane 9. Control $\Delta I507/N$, lane 10.

Figure 3 Detection of $\Delta F508$ and $\Delta I507$ mutations. PAGE of PCR amplified DNA samples using primers which flank the $\Delta F508$ and $\Delta I507$ mutant sites. Lanes 1 to 5 are members of a mild CF family and lane 6 is mild CF adult patient. Control $\Delta F508/\Delta F508$ showing a 95 bp fragment, lane 7. Control N/N showing a 98 bp fragment, lane 8. Control $\Delta F508/N$ showing both 98 and 95 bp fragments and two characteristic slow running heteroduplex bands, lane 9. Control $\Delta I507/N$ showing both 98 and 95 bp fragments and a single heteroduplex band, lane 10.

As shown in fig 3, three members of the family were heterozygous for the G551D mutation. These were the father (I·1), son (II·1), and older daughter (II·2). However, the mother (I·2) and younger daughter (II·3) had normal alleles at all three of the possible mutant sites tested.

The unrelated CF affected female had a single G551D mutation as shown in fig 1, and was also heterozygous for the $\Delta I507$ deletion (fig 3). Owing to some cross reaction with the $\Delta F508$ hybridisation oligonucleotide (fig 2), it is possible to misinterpret the $\Delta I507$ mutation as $\Delta F508$. However, as shown in fig 3, when compared with the controls on a polyacrylamide gel, the pattern of the amplified DNA from this patient is clearly that of a $\Delta I507$ and not a $\Delta F508$ heterozygote.

Discussion

A large combined European study³ has shown that the major CF mutation, $\Delta F508$, is present on 70% of defective CF chromosomes. However, the frequency of $\Delta F508$ increases in a south-east to north-west direction. Lower frequencies are observed in southern populations, with a minimum value of 27% in Turkey which rises to a maximum of 88% in Denmark. The British contribution to this study from several centres indicates a frequency for $\Delta F508$ of 75% in this country.³

The family with one normal child and two children with an apparently mild CF-like phenotype were referred to the Northern Region Genetic Advisory Service for DNA studies as a possible method of explaining the phenotype and thus confirming the diagnosis. As shown in fig 2, it was impossible to make this confirmation based on the initial screen for the presence of the $\Delta F508$ deletion in this family, even though the expectation was to detect it on at least one chromosome 7 in 90% of CF patients.²

A summary of the combined European data³ shows that 50% of CF patients suffering from pancreatic insufficiency are homozygous for $\Delta F508$ and a further 36% are heterozygous for the deletion at this site. Of those patients with a history of meconium ileus, 58% are homozygous and a further 29% are heterozygous for this common mutation. This suggests that about 85% of severely affected subjects have the $\Delta F508$ mutation on at least one of their two defective CF chromosomes.

The possible phenotypic effect of the non- $\Delta F508$ mutations must also be considered. Ninety percent of patients who are pancreatic sufficient have at least one of the less frequent mutations: 43% are compound heterozygotes $\Delta F508$ /non- $\Delta F508$ and 47% do not have the $\Delta F508$ deletion at all.³

These figures would support the model proposed by Kerem *et al*² which suggests that $\Delta F508$ is a severe allele which is recessive to all mild alleles, and that a second severe allele must exist to account for severely affected patients who have a single copy, or no copies, of the $\Delta F508$ deletion. However, the model also predicts that there should be no mild cases in the $\Delta F508/\Delta F508$ category. To account for the small number of patients in the latter category, the authors of the European study suggest possible misdiagnosis, though the emerging situation is complex and may remain so with the identification of more mutant alleles and correlation with various phenotypes.

Further detailed studies on the family described here detected the less frequent G551D mutation to be present in the two CF-like children (II·1 and II·2) but not in the normal child (II·3). This defective allele has been inherited from the father (I·1). Therefore, the children, II·1 and II·2, do have at least one defective CF gene and appear to be suffering from a mild form of cystic fibrosis which may be specifically

related to a particular combination of non- $\Delta F508$ mutations. If this is the case, then they will be compound heterozygotes for an as yet unidentified mutation inherited from their mother (I-2). An alternative explanation is that G551D gives rise to a more severe form of the disorder, perhaps lethal at a very early developmental stage when homozygous, such that these two cases are manifesting CF carriers. However, the apparent lack of CF symptoms in the father (I-1) would tend to argue against this hypothesis, and against the hypothesis that G551D could sometimes manifest itself as a dominant CF mutation.

In addition, the unrelated adult female CF patient described here has been shown to have two defective CF genes, one with the G551D mutation and one with $\Delta I507$, and she also shows mild symptoms. The apparent association of $\Delta I507$ with a mild phenotype further illustrates the precise influence different mutations in the CF gene may have on the functioning of the CFTR protein. $\Delta F508$ and $\Delta I507$ are at adjacent sites suggesting that removal of phenylalanine disrupts the ATP binding domain to a greater extent than the removal of isoleucine.

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