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The incidence of different cystic fibrosis mutations in the Scottish population: effects on prenatal diagnosis and genetic counselling

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Abstract

We present an analysis of the frequency of 16 different cystic fibrosis (CF) mutant alleles in the Scottish population. Each allele was detected in DNA amplified by the polymerase chain reaction (PCR) either directly on polyacrylamide gels, on agarose gels after restriction enzyme digestion, or by using allele specific oligonucleotides. Among 506 CF chromosomes, of predominantly Scottish origin, the frequencies of the different mutations were △F508 0·71, G551D 0·05, G542X 0·04, R117H 0·01, 1717-1G→A 0·01, A455E +△I507 + R553X + R560T + W1282X + 621+1G→T combined 0·03, unpublished 0·01, and unknown 0·13. No examples of D110H, R347P, S549N, S549I, or 2566ins AT mutations were found. The relevance of this type of analysis for both prenatal diagnosis and heterozygote screening is discussed.

Cystic fibrosis (CF) is the most common, serious, recessively inherited disorder among Caucasians at 1 in 2500 births. Recent data from the USA suggest a median life expectancy of 27 years.[1] The gene defective in CF has recently been cloned and shown to encode a 1480 amino acid, 168 kd protein named cystic fibrosis transmembrane conductance regulator (CFTR).[2] The protein shows homology to a family of proteins with unidirectional transportation properties which require ATP hydrolysis and is thought to be involved in the regulation of chloride transport rather than functioning as a chloride channel per se.[2 3] Expression of CFTR cDNA in epithelial cells from CF patients restores the normal regulation of chloride ion transport.[4 5]

Over 50 putative cystic fibrosis mutations have been identified and a nomenclature devised using the single letter amino acid code. For example the R117H mutation[6] involves an arginine to histidine change at position 117, whereas △F508 represents the deletion of an amino acid, phenylalanine, at position 508. Examination of the available data shows that there appears to be a cluster of mutations in the first nucleotide binding fold.[7 8]

The first mutation described in CFTR, △F508,[9] is present at different frequencies in populations of different ethnic origin.[10] It is therefore likely that other rarer mutations will also be present at varying frequencies in different populations. A knowledge of this variation is required for accurate genetic counselling and for population based heterozygote screening. We have therefore analysed an extensive cohort of Caucasian patients attending cystic fibrosis clinics in and around Edinburgh (and in many cases their immediate families) and a number of families referred to us for prenatal diagnosis through the Scottish Molecular Genetics Consortium[11] for the mutations in CFTR described in published reports.[6-9 12-15] A small number of families from England was also analysed.

Searching for common CFTR mutations allows one to modify the population risk of a subject being a CF carrier. This is a growing area in genetic counselling when cystic fibrosis is known in the family and we present data on the analysis of the partners of CF carriers.

Methods

POLYMERASE CHAIN REACTION (PCR) AND ANALYSIS

Genomic DNA was extracted from whole blood by standard methods or by use of an Applied Biosystems Model 340A DNA Extractor and amplified using 0·5 to 2 units of AmpliTaq(TM) (Perkin-Elmer/Cetus, Hemel Hempstead) in 50 to 100 µl of 50 mmol/l KCl, 2·5 mmol/l $MgCl_2$, 10 mmol/l Tris-Cl (pH 8·3 at 25°C) under the conditions shown in table 1A. Where necessary restriction digests were performed on 10 µl of PCR product under the conditions recommended by the supplier and then electrophoresed in 2% agarose, 0·5×Tris-borate/EDTA (TBE) gels. The △F508 and △I507 mutations were visualised directly

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Table 1 Conditions used for the detection of defined CF mutations. (A) PCR primers, annealing temperatures, and restriction enzymes. (B) Allele specific oligonucleotides and hybridisation temperatures (other conditions as described[16]).

A Primer sequence	Annealing temp (°C)	Mutation	Detection method
Exon 4			
5′ AGTCACCAAAGCAGTACAGC	50	R117H	ASO
5′ GCTATTCTCATCTGCATTCC		D110H	ASO
5′ TCACATATGGTATGACCCTC (a)	50	621+1G→T	RFLP *Mse*I
5′ TTGTACCAGCTCACTACCTA (a)			
Exon 7			
5′ CAGAACTGAAACTGACTCGG	50	R347P	RFLP *Msp*I
5′ TGCTCCAAGAGAGTCATACC			
Exon 9			
5′ TAATGGATCATGGGCCATGT (a)	58	A455E	ASO
5′ ACAGTGTTGAATGTGGTGCA (a)			
Exon 10			
5′ GTTTTCCTGGATTATGCCTGGCAC	58	ΔF508	10% PAGE
5′ GTTGGCATGCTTTGATGACGCTTC		ΔI507	
Exon 11			
		1717–1G→A	ASO
		G542X	ASO
5′ TTCAGCAATGTTGTTTTGACCAAC	52	S549N	RFLP *Dde*I
5′ CACAGATTCTGAGTAACCATAATC		S549I	RFLP *Dde*I
		G551D	RFLP *Hinc*II + *Mbo*I
		R553X	RFLP *Hinc*II
		R560T	ASO
Exon 13			
5′ TGTGTCTGTAAACTGATGGCTAACAA (b)	50	2566insAT	10% PAGE
5′ TCTTCGTTAATTTCTTCACTTATTTC (b)			
Exon 20			
5′ GGTCAGGATTGAAAGTGTGCA (a)	55	W1282X	ASO
5′ CTATGAGAAAACTGCACTGGA (a)			

B Mutation	Oligonucleotide		Hybridisation temp (°C)
D110H	5′ TCCTATCACCCGGAT	(c)	48
R117H	5′ GAGGAACACTCTATC	(c)	48
Normal exon 9	5′ GTTGTTGGCGGTTGCT	(d)	52
A455E	5′ GTTGTTGGAGGTTGCT	(d)	52
1717–1G→A	5′ TGGTAATAAGACATCTC		47
Normal exon 11	5′ ACCTTCTCCAAGAACT	(e)	46
G542X	5′ ACCTTCTCAAAGAACT	(e)	46
R560T	5′ CTTTAGCAACGTGAATAAC	(f)	50
Normal exon 20	5′ CAACAGTGGAGGAAAGCCTT	(a)	56
W1282X	5′ CAACAGTGAAGGAAAGCCTT	(a)	56

Unless otherwise stated, all oligonucleotides were obtained from OSWEL DNA Service (Edinburgh). Other sources: (a) European Community Cystic Fibrosis Consortium. (b) Dr J Dorin. (c) Dr M Dean. (d) Dr B-S Kerem. (e) Dr M Schwartz. (f) Mr M Schwartz.

Table 2 Conditions used for the analysis of CF linked RFLPs by PCR.

Marker	Primers	Annealing temp (°C)	Enzyme	Reference
XV–2c	5′ GTTGAAGTGAATTGAATG 5′ TGAGTCTCTGCTGCCAGT*	52	*Taq*I	19
KM19	5′ TGCATCATATAAGTTGCC 5′ GGCTACACTGTTAATTTT	40	*Pst*I	17
J3.11	5′ AGCACACTAGGGATGTTC 5′ GGCAAATAGAAACAGAGG	50	*Msp*I	17
MP6d–9	5′ AATGCAACAATTCACCCAATTGCTCA 5′ GGTTAGGTCAGAGAACAAAGCAAATT	47	*Msp*I	20

*Beaudet, personal communication.

by electrophoresis in 18 cm 10% polyacrylamide (29:1 acrylamide:bis-acrylamide) gels. Gels were run at 30 mA for 2·5 hours (0·5×TBE) or 4 hours (1×TBE), and stained in 2 μg/ml ethidium bromide.

Hybridisations with the allele specific oligonucleotides shown were performed as previously described[16] except that Hybond N^+ membranes (Amersham) were used. The oligonucleotides and hybridisation temperatures used are shown in table 1B. Whenever possible we analysed DNA from both parents as well as the index case to show that the mutations under study were on separate chromosomes. This has the additional benefit of supporting paternity. Cystic fibrosis linked polymorphisms were also analysed by PCR amplification (table 2). On all samples the XV-2c (*TaqI*) and KM19 (*PstI*) genotypes were analysed in order to determine where possible the XV-2c/KM19 haplotypes of the CF chromosomes. We also analysed J3.11 (*MspI*) and MP6d-9 (*MspI*) when required to identify cystic fibrosis chromosomes.

Results and discussion

MUTATION ANALYSIS

In a previous report we showed that the exon 10 ΔF508 mutation accounts for 73% of CF chromosomes in complete families in the Scottish population.[17] Thus, in 53% of families the index case is expected to have inherited this allele from both parents. In the present study we have looked at a larger sample population, of predominantly Scottish origin, and the ΔF508 frequency is 71% (51% of index cases having two copies of ΔF508). There was no significant difference between the Scottish and English populations analysed (306/430 Scottish=71·2%; 55/76 English=71·3%). Owing to the smaller number of chromosomes in the English sample it is not possible to compare the relative frequencies of the other, rarer mutations described below. In the following discussion, figures are a percentage of all 506 chromosomes analysed (table 3).

Using polyacrylamide gel electrophoresis (PAGE) it is possible to discriminate between ΔF508 and the neighbouring deletion ΔI507.[8 12] The heteroduplexes formed between the 95 bp and 98 bp products in the latter stages of PCR migrate through polyacrylamide gels in a conformation dependent manner. The sequence difference between the two deleted products (deletion of ATC/GAT or CTT/AGG) results in different heteroduplex conformations and hence different rates of migration in the gel (figure). ΔI507 is a rare mutation which we have observed on three apparently unrelated CF chromosomes.

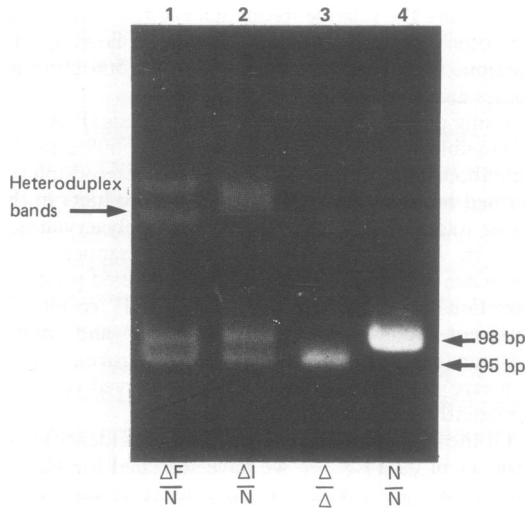
Of the nine individual point mutations identified in exon 11 of CFTR,[7 8 12] we have searched for six. Of these, two are present at significant levels in the samples analysed (table 3). G551D is detected by digestion with restriction enzymes *Hin*cII and *Mbo*I. The G→A transition at nucleotide 1704 removes a *Hin*cII site and creates an *Mbo*I site. G542X is detected by the use of an allele specific oligonucleotide (ASO). Three separate mutations affect the serine residue at codon 549. Two of these (S549N and S549I) destroy a *Dde*I site. We have not observed any chromosomes carrying these mutations. R553X destroys the same *Hin*cII site as G551D but does not create another restriction site. The clustering of missense mutations in a putative ATP binding fold indicates the functional importance of this region.[3 7 8]

Using a combination of chemical modification and direct sequencing (C T Jones *et al*, in preparation), we identified two putative splice mutations, namely 1717-1G→A and R560T, which had been described previously.[8 12 15] We subsequently analysed these

Table 3 Mutations identified on CF chromosomes from index cases in a predominantly Scottish population.

Mutation	Exon	Haplotype	No	%	Reference
D110H	4	—	0	0	6
R117H	4	C	7	1·4	6
621+1G→T	*	B	3	0·6	12
R347P	7	—	0	0	6
A455E	9	B	1	0·2	8, 12
ΔI507	10	D	3	0·6	8, 12
ΔF508	10	B	361	71·3	8, 9
1717-1G→A	†	B	5	1·0	8, 12, 15
G542X	11	B	20	4·0	8, 12
S549N	11	—	0	0	7
S549I	11	—	0	0	8, 12
G551D	11	B	28	5·5	7
R553X	11	A	1	0·2	7
R560T	11	D	3	0·6	8, 12
2566insAT	13	—	0	0	13
W1282X	20	B	2	0·4	8, 14
Unpublished			7	1·4	
Unknown			65	12·8	
Total			506		

*=intron 4. †=intron 10. —=not determined.
Haplotypes shown are defined by alleles at the XV-2c and KM19 loci, as follows: A 2·1 kb, 7·8 kb; B 2·1 kb, 6·6 kb; C 1·4 kb, 7·8 kb; D 1·4 kb, 6·6 kb, and represent the background on which each mutation was most often observed.



Ethidium bromide stained 10% polyacrylamide gel showing the direct detection of ΔF508 and ΔI507. The samples are from a ΔF508 heterozygote (lane 1), a ΔI507 heterozygote (lane 2), a ΔF508 homozygote (lane 3), and a non-deletion homozygote (lane 4).

using ASO hybridisation and found each represents approximately 1% of CF chromosomes in the study population.

A number of mutations have been described in the first transmembrane region of CFTR, which appear to be associated with a milder phenotype.[6] Using an allele specific oligonucleotide (gift of M Dean) we have shown one of these, R117H, to be present on approximately 1% of CF chromosomes in this study. We did not observe either D110H or R347P detectable by allele specific oligonucleotides and *Msp*I digestion, respectively. Three examples of the 621+1G→T intron 4 splicing mutation were also detected.[12] Other mutations identified in the populations studied are shown in table 3. The XV-2c/KM19 haplotype most commonly associated with each mutation identified is also shown. It is clear that the linkage disequilibrium observed between the 'B' haplotype (XV-2c/2·1 kb; KM19/6·6 kb) and the CF locus is not the result of linkage to a single mutant allele. A possible explanation of the recurrence of CF mutations on the same haplotype involves a combination of hitchhiking and epistasis.[18]

Four of the mutations we identified are only known to us through the Cystic Fibrosis Genetic Analysis Consortium and cannot be described in detail in this communication. A detailed comparison of genotype and phenotype in the patients studied here will be published separately.

In cases where one or both CFTR mutations await identification, all families analysed can be made fully informative using polymorphic markers linked to the CF locus.

Table 4 Prenatal diagnoses between September 1989 and October 1990.

ΔF508/ΔF508	11
ΔF508/G551D	2
ΔF508/R117H	1
ΔF508/other*	7
Total	21

*In these cases the other CF allele was tracked using linked markers.

PRENATAL DIAGNOSIS

We have performed 21 prenatal diagnoses (table 4), using direct analysis of CFTR mutations, at first using ΔF508 and then G551D and R117H (as they were identified). All families have been screened for ΔF508, G551D, G542X, and R117H, while most have been screened for each of the CF mutations described in this report.

Chromosomes with unidentified CF mutations were followed with CF linked markers shown to be segregating with the mutation. As more CF mutations have been described the proportion of prenatal diagnoses using both mutations rather than CF linked polymorphisms has increased over the first year. At present over 85% of families are fully informative for both CF mutations segregating. (In 137 CF families the index case and both parents were available for analysis. In this subset of the population we have been able to identify 92·5% of CF mutations, hence the figure of 85%.)

GENETIC COUNSELLING

The identification of over 85% of the cystic fibrosis mutations on all CF chromosomes analysed (table 3) has resulted in an increase in workload for the counselling services. In the first instance, the relatives of CF patients and known carriers request carrier status determination. The partners of known carriers subsequently request analysis to determine whether they also carry a CF mutation.

From the data in table 3, it appears that ΔF508, G551D, G542X, and perhaps R117H are sufficiently common to justify analysis in the partners of CF heterozygotes identified in family studies. Exclusion of these four mutations would reduce a subject's risk from 1:25 to 1:136, and hence the apparent risk to the pregnancy from 1:100 to 1:543. Analysis for all mutations detected in this population to date reduces these figures to 1:189 and 1:754, respectively. We have analysed 53 partners of known CF heterozygotes and identified four subjects heterozygous for ΔF508 and one heterozygous for R117H. This latter observation is particularly interesting since the data in table 3 would predict an overall frequency of 1:1785 for this allele in the population.

Although the population in this study was of largely Scottish origin, the findings are expected to be

generally applicable to other United Kingdom populations. It will be interesting to compare similar data from other parts of northern Europe.

RECOMBINATION/CARRIER STATUS

It has become more straightforward and informative to follow CF chromosomes from nuclear families into second degree relatives using the CF mutations rather than by CF linked markers, as there is no increased error owing to recombination. An increased proportion of our families are now being analysed in this way.

To eliminate the possibility of recombination, subjects who had previously been tested for their carrier status with linked markers were analysed once more for the mutation(s) identified in each family. In only one of 78 families was a recombinant between CFTR and linked marker (pJ3.11) identified, but with profound effect to the carrier status assignments within the family. Four subjects were now found to be carriers, having been given a low risk of being carriers by linked marker analysis. One of the subjects whose status was changed was found to have a partner who carried $\triangle$F508.

Unaffected sibs of CF index cases with both mutations identified can be screened for their carrier status, and our finding of 39 out of 61 CF carriers was close to the expected two thirds. However there was a just significant (50 of 81:$0{\cdot}05>p>0{\cdot}02$) excess of CF carriers among sibs of known carriers of identified mutations.

NON-PATERNITY

Out of 100 families in which both cystic fibrosis mutations had been identified, one case of previously undiscovered non-paternity was identified by finding that the 'father' did not carry the appropriate CF mutation found in the index case. This had not been detected earlier despite the family having been studied with nine CF linked polymorphisms. Non-paternity was confirmed by DNA fingerprint analysis by our colleagues in Aberdeen.

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1 Boat TF, Welsh MJ, Beaudet AL. Cystic fibrosis. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. *The metabolic basis of inherited disease*. 6th ed. New York: McGraw-Hill, 1989: 2649–80.
2 Riordan JR, Rommens JM, Kerem BS, *et al.* Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science* 1989;**245**:1066–73.
3 Hyde SC, Emsley P, Hartshorn MJ, *et al.* Structural model of ATP-binding associated with cystic fibrosis, multidrug resistance and bacterial transport. *Nature* 1990;**346**:362–5.
4 Drum ML, Pope HA, Cliff WH, *et al.* Correction of the cystic fibrosis defect in vitro by retrovirus mediated gene transfer. *Cell* 1990;**62**:1227–33.
5 Rich DP, Anderson MP, Gregory RJ, *et al.* Expression of cystic fibrosis transmembrane conductance regulator corrects defective chloride channel regulation in cystic fibrosis epithelial cells. *Nature* 1990;**347**:358–63.
6 Dean M, Amos J, Hsu JMC, *et al.* Multiple mutations in highly conserved residues are found in mildly affected cystic fibrosis patients. *Cell* 1990;**61**:863–70.
7 Cutting GR, Kasch LM, Rosenstein BJ, *et al.* A cluster of cystic fibrosis mutations in the first nucleotide-binding fold of the cystic fibrosis conductance regulator protein. *Nature* 1990;**346**: 366–9.
8 Kerem BS, Zielenski J, Markiewicz D, *et al.* Identification of mutations in regions corresponding to the 2 putative nucleotide (ATP)-binding folds in the cystic fibrosis gene. *Proc Natl Acad Sci USA* 1990;**87**:8447–51.
9 Kerem BS, Rommens JM, Buchanan JA, *et al.* Identification of the cystic fibrosis gene: genetic analysis. *Science* 1989;**245**: 1074–80.
10 Cystic Fibrosis Genetic Analysis Consortium. Worldwide survey of the $\triangle$F508 mutation. Report from the cystic fibrosis genetic analysis consortium. *Am J Hum Genet* 1990;**47**:354–9.
11 Brock DJH. A consortium approach to molecular genetic services. *J Med Genet* 1990;**27**:8–13.
12 Kerem B, Bozon D, Zielenski J, *et al.* Identification of mutations in the cystic fibrosis gene. *Am J Hum Genet* 1990;**47**:223A.
13 White MB, Amos J, Hsu JMC, *et al.* A frameshift mutation in the cystic fibrosis gene. *Nature* 1990;**344**:665–7.
14 Vidaud M, Fanen P, Martin J, Ghanem N, Nicolas S, Goossens M. Three point mutations in the CFTR gene in French cystic fibrosis patients: identification by denaturing gradient gel electrophoresis. *Hum Genet* 1990;**85**:446–9.
15 Guillermit H, Fanen P, Ferec C. A 3′ splice site consensus sequence mutation in the cystic fibrosis gene. *Hum Genet* 1990;**85**:450–3.
16 Brind AM, McIntosh I, Brock DJH, James OFW, Bassendine MF. Polymerase chain reaction for detection of the alpha-1-antitrypsin Z allele in chronic liver disease. *J Hepatol* 1990;**10**: 240–3.
17 McIntosh I, Curtis A, Lorenzo ML, *et al.* The haplotype distribution of the $\triangle$F508 mutation in cystic fibrosis families in Scotland. *Hum Genet* 1990;**85**:419–20.
18 Wagner DK, Cavalli-Sforza LL. Ethnic variation in genetic disease: possible roles of hitchhiking and epistasis. *Am J Hum Genet* 1975;**27**:348–64.
19 Rosenbloom CR, Kerem BS, Rommens JM, *et al.* DNA amplification for detection of the XV–2c polymorphism linked to cystic fibrosis. *Nucleic Acids Res* 1989;**17**:7117.
20 Huth A, Estivill X, Grade K, *et al.* Polymerase chain reaction for the detection of the pMP6–9/*Msp*I RFLP, a marker closely linked to the cystic fibrosis mutation. *Nucleic Acids Res* 1989;**17**:7118.