Mutation analysis of 184 cystic fibrosis families in Wales

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Abstract

We describe a molecular analysis of 184 cystic fibrosis (CF) families in Wales. To determine accurate frequency data for the CF mutations in the Welsh population, families with at least three Welsh grandparents were strictly regarded as Welsh. Of these 74 families, we have identified approximately 90% of mutations causing CF, with $\Delta F508$ accounting for 71.8% and $621+1G>T$ 6.7%. We observed a significant difference between the Welsh and Scottish frequencies of $621+1G>T$.

To allow the rapid and efficient screening for the more common mutations we modified a multiplex used by Watson *et al* enabling the detection of $\Delta F508$, G551D, and R553X simultaneously with $621+1G>T$. In parallel to this system we ran the Cellmark Diagnostics ARMS multiplex kit, which detects $\Delta F508$, $621+1G>T$, G551D, and G542X.

RFLP analysis of the 184 families shows that the $\Delta F508$ chromosomes are almost exclusively found on the B haplotype (XV2c 1, KM19 2); the other CF mutations have more heterogeneous backgrounds. Strong haplotype correlations exist between the markers XV2c, KM19, D9, and G2 and the other CF mutations. Haplotype data suggest that there are at least seven mutations that remain to be identified in these families.

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In 1989 the cystic fibrosis transmembrane conductance regulator (CFTR) gene and the most common mutation, $\Delta F508$, were identified.¹⁻³ Marked variations in the frequency of $\Delta F508$ have been reported from different populations.⁴ For example, in different regions of Europe,

$\Delta F508$ varies from as low as 30% to as high as 80%.⁵ A large number of other mutations have also been reported in the CFTR gene,⁶⁻¹⁵ although the majority have been identified only in individual cases. From a study of 184 cystic fibrosis families referred to our centre, we have established the frequency of the $\Delta F508$ mutation and many of the rarer mutations in the Welsh population. Additionally we have performed extensive RFLP analysis particularly on the non- $\Delta F508$ CF chromosomes and established haplotype correlations with the other mutations. From these studies we have predicted the number of mutations that remain unidentified in our total population.

Materials and methods

In a study of 184 CF families, 370 normal chromosomes and 369 CF chromosomes (one shared between first cousins) were analysed. Although 98% of these families currently reside in Wales, only families with at least three Welsh grandparents (74 in total) were strictly regarded as Welsh for the attainment of accurate frequency data. Of the remaining 110 families, 63 had two or fewer Welsh grandparents (the others being of British origin), and in the other 47 families the nationalities of the grandparents were undefined. The diagnosis of CF was made on the basis of abnormal sweat electrolyte concentrations and clinical findings. Screening for $\Delta F508$ and 16 other rarer CF mutations was carried out by the procedures described by the original investigators.

Multiplexes permit the co-amplification of several primer sets, thus generating multiple products for analysis. To allow the rapid and efficient screening of the more frequent mutations two multiplexes were used. A multiplex test based on the Amplification Refractory Mutation System (ARMS), developed by Cellmark Diagnostics,¹⁶ allows the simultaneous detection of $\Delta F508$, G551D, G542X, and $621+1G>T$. The test comprises two ARMS reactions. The A reaction identifies normal $621+1G>T/\Delta F508$ sequences and mutant G551D/G542X sequences, whereas the B reaction identifies mutant $621+1G>T/\Delta F508$ sequences and normal G551D/G542X sequences (fig 1).

In parallel to this system we ran a modified version of the multiplex used by Watson *et al*,¹⁷ which subsequently allowed $621+1G>T$ to be screened with $\Delta F508$, $\Delta I507$, G551D, and R553X at the same time. We adopted the name 'Cardiff Multiplex' (CMP) for this modified system. The exon 10 (C16B and C16D) primers² were co-amplified with exon 11 primers,¹⁸ and a set of modified exon 4 primers (4e5' and 4i3'). A

Figure 1 The ARMS multiplex analysed on a 3% NuSieve gel. Samples 1 to 3 are normal, 4 is a $\Delta F508$ heterozygote, 5 a G542X/ $\Delta F508$, 6 a G551D/ $\Delta F508$, 7 a $621+1G>T/\Delta F508$, and 8 a G551D homozygote.

Figure 2 The Cardiff multiplex analysed on a 10% polyacrylamide gel. Sample 1 is normal, 2 a $\Delta F508$ heterozygote, 3 a $\Delta I507$ heterozygote, 4 a $G551D$ or $R553X$ heterozygote, and 5 a $621 + 1G > T$ heterozygote.

HincII digestion and analysis on a 10% polyacrylamide gel detects $\Delta F508$, $\Delta I507$, $G551D$, and $R553X$ (fig 2), and an *MseI* digest followed by analysis on a 3% NuSieve gel detects $621 + 1G > T$ (fig 3). PCR was carried out in 50 μ l reaction mixes containing 100 to 500 ng of genomic DNA, 10 pmol of the C16 primers, 20 pmol of the exon 11 primers, 35 pmol of 4e5' and 4i3', 0.2 mmol/l of each dNTP, 5 μ l of Cetus buffer (100 mmol/l Tris-HCl, pH 8.3, 500 mmol/l KCl, 15 mmol/l MgCl₂, 0.01% gelatin), and 1 unit of Cetus *Taq* polymerase. The cycling parameters were an initial denaturation at 94°C for 10 minutes, followed by 30 cycles of 56°C for one minute, 72°C for one minute, 93°C for 30 seconds, and a final step of 56°C for one minute and 72°C for 10 minutes. The sequence of the 4e5' primer is 5'-CGA TTT ATC TAG GCA TAG GC-3'.

Restriction fragment length polymorphism (RFLP) analysis was by standard methods. The majority of the CF chromosomes were typed for XV2c and KM19, and more extensive haplotyping (MetD, MetH, G2, D9, and

J3.11) was performed on the non- $\Delta F508$ CF chromosomes.

The classification of pancreatic insufficiency (PI) or pancreatic sufficiency (PS) was made depending on whether the patient needed enzyme supplementation or not (all those regarded as PS had a three day faecal fat excretion test). Where there was uncertainty or if it was not possible to make a diagnosis, the patient was classified as P?.

Results

The frequencies of the CF mutations studied to date in the 184 families are presented in table 1. We have identified a total of 89.8% of CF mutations in the Welsh population, with $\Delta F508$ accounting for 71.8%, $621 + 1G > T$ 6.7%, $G542X$ 2.7%, and $R1283M$ 2.0%. The differences in the numbers of each mutation between the Welsh and the mixed groups were relatively small and not statistically significant. To ensure that the mixed group was not disguising any significant differences, since it contained some families with one or two Welsh grandparents, for each individual chromosome the probability of it being Welsh was then calculated. When two groups were divided into Welsh and not Welsh (each chromosome could count as partially Welsh and would contribute accordingly to the Welsh total, and vice versa), no significant differences were seen. For subsequent haplotype analysis the data sets were thus pooled.

A comparison of the numbers of each mutation in the Welsh group and the Scottish population¹⁹ was carried out. Expected numbers for each mutation in the different groups were calculated, and the mutations with expected numbers less than 2.5 in the Welsh or Scottish groups were combined into a single category of rare mutations. $\Delta F508$ was not included in the analysis, as it was already known to have a fairly uniform frequency within the British population.⁵ Statistical analysis involved a χ^2 analysis with 4 degrees of freedom (df). Results showed a χ^2 value of 27.2 which is the equivalent to a p value < 0.0005 . The difference in frequency of $621 + 1G > T$ was almost solely responsible for these significant findings. When this mutation alone was compared to all others combined, a χ^2 of 23.8 was obtained (1 df, $p < 0.000001$).

Table 2 shows the distribution of genotypes in the 185 patients in this study. A detailed genotype/phenotype analysis will be published elsewhere (L Al-Jader, personal communication); however, it is interesting to note here that none of the pancreatic sufficient patients are homozygous for $\Delta F508$. In general we have very few pancreatic sufficient patients in our total population; however, a more detailed examination of our unclassified group may slightly alter this distribution.

From table 3 it can be seen that all but one of the $\Delta F508$ chromosomes are associated with the B haplotype, the exception being on the D haplotype. The other CF mutations have more heterogeneous backgrounds, although the majority are still associated with the B haplotype, followed by the C haplotype. Normal chromo-

Figure 3 The Cardiff multiplex analysed on a 3% NuSieve gel. Samples 1 to 5 are described in the legend to fig 2.

Table 1 Frequencies of CF mutations in our study. 'Welsh' families are defined as those with three or more Welsh grandparents and 'mixed' families have two or fewer Welsh grandparents.

Mutation	Welsh		Mixed		Undefined		Total	
	No	%	No	%	No	%	No	%
ΔF508	107/149	71.8	92/126	73.0	69/94	73.4	268/369	72.6
621+1G>T	10/42*	6.7	5/34*	4.0	4/25*	4.3	19/101*	5.1
G551D	2/42*	1.3	6/34*	4.8	3/25*	3.2	11/101*	3.0
G542X	4/42*	2.7	4/34*	3.2	1/25*	1.1	9/101*	2.4
G85E	0/41*	0.0	2/34*	1.6	3/24*	3.4	5/99*	1.4
R553X	2/42*	1.3	2/34*	1.6	0/25*	0.0	4/101*	1.1
R1283M	3/42*	2.0	0/34*	0.0	0/25*	0.0	3/101*	0.8
N1303K	1/42*	0.7	1/34*	0.8	0/24*	0.0	2/100*	0.6
ΔI507	2/149	1.3	0/126	0.0	0/94	0.0	2/369	0.5
R117H	1/42*	0.7	1/34*	0.8	0/25*	0.0	2/101*	0.5
1717-1G>A	2/42*	1.3	0/34*	0.0	0/25*	0.0	2/101*	0.5
R560T	0/42*	0.0	0/34*	0.0	1/25*	1.1	1/101*	0.3
1154InsTC	0/40*	0.0	1/33*	0.9	0/24*	0.0	1/97*	0.3
V520F	0/42*	0.0	0/34*	0.0	0/25*	0.0	0/101*	0.0
W1282X	0/42*	0.0	0/34*	0.0	0/25*	0.0	0/101*	0.0
R347P	0/42*	0.0	0/34*	0.0	0/24*	0.0	0/100*	0.0
Q493X	0/42*	0.0	0/34*	0.0	0/24*	0.0	0/100*	0.0
Total (%)		89.8		90.7		86.5		89.1

* Non-ΔF508 chromosomes.

somes carry mainly the A or C haplotypes.

Extensive RFLP analysis on the non-ΔF508 CF chromosomes (table 4) shows that with the markers XV2c, KM19, D9, and G2, strong haplotype associations with the other mutations can be observed. For example 621+1G>T is found exclusively on the 1,2,2,2 haplotype, G551D is exclusively on the 1,2,2,1 haplotype, and R1283M is always on the 2,2,2,1 haplotype. R553X, however, is associated with two haplotypes (2,1,1,1 and 1,1,2,2) which is consistent with the hypothesis that this is a recurrent mutation.²⁰

Using this correlation, we have attempted to determine the number of mutations that remain unidentified in our total population. Through classifying the remaining 40 unidentified CF chromosomes according to their XV2c, KM19, G2, and D9 haplotypes, at least seven different groups can be established (table 5). The chromosomes with incomplete

haplotypes have been assigned to their most likely groups, but it is possible that they may belong to new groups.

Discussion

This report shows that ΔF508 accounts for 71.8% of CF chromosomes in the Welsh population. This figure is in accordance with other frequencies quoted for the major mutation within the British²¹ and Scottish²² populations (71.5% and 73% respectively). Unexpectedly we observed an unusually high frequency for 621+1G>T, 6.7%, which is significantly higher than the Scottish frequency (0.6%). Zielenski *et al*⁶ previously reported an abundance of this mutation in the French-Canadian population, with all the chromosomes carrying this mutation belonging to the haplotype group Ib. All of our 621+1G>T chromosomes also belong to group Ib, thereby substantiating this correlation.

A variety of methods for the analysis of multiple cystic fibrosis mutations has been presented.²³ After recognising a relatively high frequency of 621+1G>T in the Welsh population, we modified a multiplex used by Watson *et al*¹⁷ to allow the simultaneous detection of this mutation with ΔF508, ΔI507, G551D, and R553X; 82.3% of our total CF chromosomes can thus be screened using this system. In parallel to this we ran the ARMS multiplex which detects ΔF508, G551D, G542X, and 621+1G>T, thus accounting for 83.1% of our total CF chromosomes. Both systems have proved reliable in our hands and the ARMS multiplex is particularly robust.

The distribution of genotypes in our patients tends to support the observation that ΔF508 is a severe allele. Kerem *et al*³ suggested that patients homozygous for any combination of the severe alleles will be pancreatic insufficient, and that patients carrying either a single severe allele or two mild alleles will be pancreatic sufficient. G85E has been categorised as a severe allele by Zielenski *et al*.⁶ However, more recently, Chalkley and Harris²⁴ have reported that G85E may be associated with milder disease (that is, a mild allele). In either case, the fact that one of our five ΔF508/G85E affected subjects is pancreatic sufficient and the other four are insufficient is in conflict with the hypothesis proposed by Kerem *et al*.³

Table 2 Genotypes of the 185 patients in this study.

Pancreatic status	Genotype			Total	%
	ΔF508/ΔF508	ΔF508/other	Other/other		
PI	80	62	7	149	80.5
PS	0	4	3	7	3.8
P?	14	14	1	29	15.7
Total	94	80	11	185	
%	50.8	43.2	5.9		

Table 3 Distribution of XV2c and KM19 haplotypes in CF and normal chromosomes. For the marker loci — denotes uninformative or unavailable, 1 denotes the absence, and 2 denotes the presence of a restriction site.

Haplotype	Marker loci		CF chromosomes								Normal chromosomes			
			ΔF508				Others							
	XV2c	KM19	PI	PS	P?	Tot	(Fre) (%)	PI	PS	P?	Tot	(Fre) (%)	Tot	(Fre) (%)
A	1	1						4	1		5	(5.0)	83	22.4
B	1	2	167	3	21	191	(71.3)	36	2	3	41	(40.6)	26	7.0
C	2	1						11	2	5	18	(17.8)	88	23.8
D	2	2	1			1	(0.4)	6	2		8	(7.9)	17	4.6
A or B	1	—	9	1	7	17	(6.3)	1	1		2	(2.0)	19	5.1
C or D	2	—						2	2	4	8	(7.9)	8	2.2
A or C	—	1						9		1	10	(9.9)	10	2.7
B or D	—	2	10		3	13	(4.9)	1		1	2	(2.0)	15	4.1
A/B/C/D	—	—	35		11	46	(17.2)	5		2	7	(6.9)	95	25.7
Total			222	4	42	268		75	10	16	101		370	

Tot = total. Fre = frequency.

Table 4 Distribution of haplotypes in the chromosomes carrying the rarer CF mutations. — denotes uninformative or unavailable, 1 denotes the absence, and 2 denotes the presence of a restriction site.

Mutation	MetD TaqI	MetH TaqI	XV2c TaqI	KM19 PstI	D9 MspI	G2 XbaI	J3.11 MspI	No of chromosomes		
								PI	PS	P?
621 + 1G>T	1	2	1	2	2	2	2	9		
	1	2	1	2	2	2	1	1		
	1	2	1	2	2	2	—	1	1	
	—	2	1	2	2	2	1	1		
	1	—	1	2	2	2	2	1		
	1	—	1	2	2	2	—	1		
	—	—	1	2	2	2	2	1		
	1	—	1	2	—	2	2	1		1
	—	—	1	2	—	2	2	1		
	1	2	—	—	—	2	2	1		
Total								17	1	1
G551D	1	2	1	2	2	1	2	3		
	—	1	1	2	2	1	2	1		
	1	—	1	2	2	1	2	1		
	1	—	1	2	2	1	1	1		
	—	2	1	2	2	1	—			1
	1	—	1	2	2	—	—	1		
	—	—	1	2	2	—	2			1
	1	2	—	—	—	1	—	1		
	—	—	—	2	—	—	2	1		
								9	0	2
Total										
G542X	1	1	1	2	2	2	1	2		
	2	1	1	2	2	2	1	1		
	2	1	1	2	2	2	2	1		
	1	2	1	2	2	2	2	1		
	1	2	1	2	2	2	1	1		
	1	1	1	2	2	2	—	1		
	—	1	2	2	2	2	1	1		
	1	1	1	2	—	2	1		1	
								8	1	0
Total										
G85E	1	1	2	1	1	2	1		1	
	1	1	2	1	1	2	2	1		
	1	1	—	1	1	2	2	1		
	1	—	—	1	—	2	2	1		
	1	1	—	—	—	2	2			1
Total								3	1	1
R553X	2	1	2	1	1	1	1	1		
	1	1	1	1	2	2	2	2		
	1	1	—	1	—	2	2	1		
Total								4	0	0
R1283M	1	1	2	2	2	1	2	3		
	Total							3	0	0
N1303K	1	1	1	2	2	2	2	1		
	2	1	1	2	2	2	—	1		
Total								2	0	0
ΔI507	1	1	1	2	2	1	2	1		
	1	2	2	—	2	1	2		1	
Total								1	1	0
R117H	1	2	2	1	—	2	1		1	
	1	2	2	—	—	2	1		1	
Total								0	2	0
1717-1	1	1	1	2	2	1	2	2		
	Total							2	0	0
R560T	1	1	—	2	2	—	1			1
	Total							0	0	1
1154Ins TC	1	—	2	2	2	1	1	1		
	Total							1	0	0
								50	6	5

Haplotype analysis on disease chromosomes aids the study of the molecular defects of each of the respective mutations. Our data are consistent with the observation that the majority of ΔF508 chromosomes are characterised by the B haplotype, suggesting a common origin. In fact only one of the ΔF508 chromosomes is associated with the D haplotype, which may have arisen through a crossover event.⁵ The other CF mutations are characterised by a more heterogeneous background of haplotypes.

Strong correlations exist between the markers XV2c, KM19, D9, and G2 and the other mutations. We have categorised the remaining unidentified CF mutations according to their haplotypes with these markers. Since seven distinct haplotype groups exist, we

would predict at least seven mutations remain to be characterised in our total population; often more than one mutation exists on a single haplotype⁵ (this study). Additionally, the inability to set phase in some of the chromosomes potentially lowers the proportion of CF haplotypes, so this estimate will be on the conservative side. However, if recurrent mutations are among those that remain uncharacterised at present (these may be associated with more than one haplotype), then fewer unidentified mutations would be predicted.

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Table 5 Distribution of haplotypes in the uncharacterised CF chromosomes. — denotes uninformative or unavailable, 1 denotes the absence, and 2 denotes the presence of a restriction site.

	MetD TaqI	MetH TaqI	XV2c TaqI	KM19 PstI	D9 MspI	G2 XbaI	J3.11 MspI	No of chromosomes		
								PI	PS	P?
Group I	1	2	2	2	2	2	1		1	
Total								0	1	0
Group II	1	2	2	2	2	1	—		1	
Total	1	—	2	2	2	1	—	1	1	0
Group III	2	1	2	1	1	2	1	3		5
	2	1	2	1	1	2	2	3		
	2	1	2	1	1	2	—	1		
	1	1	2	1	1	2	1	1		
	2	1	—	1	1	2	1	2		
	2	1	—	1	1	2	—	2		
	1	—	—	1	1	2	2	1		
	—	—	—	1	1	2	2	1		
	—	1	—	1	1	2	1			1
	—	—	2	—	1	2	1			1
	2	1	2	—	1	2	1			1
	1	1	2	—	—	2	1			1
	2	1	2	—	—	2	1	1		1
	1	1	—	—	1	2	—	1		1
Total								16	0	10
Group IV	1	2	2	1	1	1	2	1		
Total								1	0	0
Group V	1	2	1	1	1	2	2		1	
	1	1	1	1	1	—	1	1		
	1	—	1	—	—	—	—	1		
Total								2	1	0
Group VI	1	2	1	1	2	2	2	1		
	1	2	1	—	2	2	1		1	
Total								1	1	0
Group VII	1	2	1	2	2	2	1	1		
Total								1	0	0
Others	2	1	2	—	—	—	—	1		
	1	—	—	—	—	2	—	1		
	—	1	—	—	—	2	1	1		
	—	—	—	—	—	—	—	—		1
Total								3	0	1
								25	4	11

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