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The Irish cystic fibrosis database

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Abstract

We have found records of 1014 Irish cystic fibrosis patients alive by December 1994, belonging to 883 families. Prevalence in the population is 1/3475 and incidence at birth 1/1461, with a gene frequency of 2·6%. Twenty percent of the patients are aged over 20 years, but at present survival rate falls rapidly after that age. We have identified 85% of the mutations on the CFTR gene in a sample of 29% of the families (506 CF chromosomes). Mutation ΔF508 is found in 72% of Irish CF chromosomes, G551D in 6·9%, and R117H in 2%. These are the highest frequencies reported for the latter two mutations world wide. Another seven mutations are found in an additional 4% of CF families. We present new microsatellite haplotype data that could be useful for genetic counselling of CF families bearing some of the 15% of CF mutations still unidentified, and comment on possible uses of our database.

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The vital statistics and mutations found in the Irish cystic fibrosis (CF) patient population are not as well described as in other European countries. For instance, the only estimate of incidence for this population was obtained by O'Reilly *et al*[1] who, searching through paediatric hospital admissions for the year 1971, found a minimal figure of 1 affected for 1800 children under 1 year of age. However, Mulherin *et al*,[2] describing their adult clinic, found that 12% of their patients had been diagnosed after the age of 10 years. With regard to the prevalence of mutations on the CFTR gene, De Arce *et al*[3] reported the deletion ΔF508 in 76% of 88 Irish CF chromosomes, but this sample is small, and many more mutations causing the disease world wide have since been identified.[4] The strong association between haplotypes for the intragenic microsatellite markers described by Morral *et al*[5] and Zielinsky *et al*[6] and specific mutations on the CFTR gene could prove particularly useful in diagnosis and in the identification of as yet unknown mutations on the CFTR gene,[7,8] and have been used as ethnic markers tracing the course of past migrations.[9,10] Here we summarise initial observations on the incidence of CF and vital statistics of these patients in Ireland, and we report on the mutations found in a sample of 506 Irish CF chromosomes and their associated haplotypes.

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Materials and methods

THE DATABASE

The database (access by Microsoft for the PC) contains three large tables prepared to accept data under the headings Demography, Genetics, and Clinical data. The information on mutations and genetic markers was obtained in the authors' laboratories from a DNA bank built up over a period of four years with the cooperation of clinical colleagues. Other statistical and clinical data relevant to the Irish CF population were obtained as follows.

FREQUENCY OF CYSTIC FIBROSIS IN IRELAND AND VITAL STATISTICS

Four sources of data were consulted: (1) Department of Health statistics on the number of persons suffering from CF and availing of the "Long Term Illness Scheme", (2) records of the Irish CF Association, (3) current hospital charts of CF patients attending any of the nine CF clinics distributed throughout the country, having obtained the necessary permission from the Consultants and local Ethics Committees, and (4) medical publications reporting on previous surveys on the Irish CF population. Population data were obtained from the 1991 Irish Census.[11] Once a comprehensive list of patients alive by December 1994 had been obtained, yearly incidence at birth was calculated retrospectively for the period 1977–1986, thus correcting for cases diagnosed after 9 years of age.

FREQUENCY OF MUTATIONS ON THE CFTR GENE

Mutation ΔF508 was screened for in an initial sample of 240 CF chromosomes by heteroduplex analysis of their PCR amplified exon 10.[12] A panel of at least 40 CF non-ΔF508 chromosomes were subsequently analysed by denaturing gradient gel electrophoresis[13] of their PCR amplified products of each of the following exons: 4, 5, 6a, 7, 8, 9, 11, 12, 14a, 15, 16, 17b, 18, 20, 21 and 23. Conditions and primers have been described previously.[14,15] This was followed by direct sequencing of the amplified products with abnormal electrophoretic patterns using the dideoxy method of Sanger *et al*.[16] After this initial work established the most common mutations, a further 266 CF chromosomes (133 CF patients) were screened for the six most common mutations using an enhanced ARMS test,[17] kindly supplied by Cellmark Diagnostics (UK).

LINKED MARKERS AND MICROSATELLITES

We also examined the linkage disequilibrium between CF mutations and three extragenic

Table 1 Age distribution of 1014 Irish CF patients alive by December 1994 compared to US

Age (y)	% Irish	% US*
0–5	12·0	22·4
6–10	22·7	22·1
11–15	22·1	17·2
16–20	22·7	13·5
21–25	11·4	10·3
26–30	5·9	7·2
31–35	2·1	4·1
36+	1·1	3·2

* Data from Fitzsimons.[21]

restriction fragment length polymorphisms (RFLPs), namely KM-19/*PstI*,[18] XV-2c/*TaqI*,[19] and Mp6-d9/*MspI*.[20] Two hundred Irish CF chromosomes and 36 non-CF chromosomes were investigated for these markers. Haplotypes for the three intragenic microsatellite markers IVS8CA,[5] IVS17bTA,[6] and IVS17bCA[6] were also investigated for a total of 109 normal, 150 CF ΔF508, and 92 CF non-ΔF508 chromosomes, using methods reported elsewhere.[7 10]

Results

FREQUENCY OF CYSTIC FIBROSIS IN IRELAND AND VITAL STATISTICS

We found records of 1014 patients alive by December 1994, belonging to 883 families; 763 of these have one affected child, 109 have two affected children, and 11 have three affected. Population data from the 1991 Census allow us to estimate prevalence at all ages to be 1 in 3475. Average yearly incidence at birth for the 10 year period 1977–1986 was 1 in 1461, with a gene frequency of 2·6%. Age distribution for the whole patient population (table 1) shows that 20% are over 20 years of age. Five of these, four women and one man, are married and have at least one child each. The genotype of three of the mothers was ΔF508/G551D, ΔF508/R117H, and ΔF508/ΔF508 respectively. The CF father is a 29 year old of genotype ΔI507/R117H. Age at death was available for 98 patients who had died after 1986, showing a lifespan of at least 20 years for 23% patients, with no significant difference between the sexes. Age at diagnosis was available for 409 patients, showing that 64% were diagnosed within their first year of life, and 18% after their third birthday. These included seven patients of genotype ΔF508/R117H, for whom the mean age at diagnosis was 13·8 years (SD 9·2 years). Two unrelated patients of genotype ΔF508/1717-1G-A were diagnosed at ages 12 and 29 years respectively, while another unrelated patient of this genotype was diagnosed at 1 month of age. The mean age at diagnosis for 59 homozygous ΔF508/ΔF508 patients was 2·3 years (SD 3·5 years).

FREQUENCY OF MUTATIONS ON THE CFTR GENE AND ASSOCIATED HAPLOTYPES

Table 2 shows the frequency of mutations found on 506 Irish CF chromosomes and their associated haplotypes. Microsatellite haplotypes found in 109 normal, 150 ΔF508, and 50 CF non-ΔF508 chromosomes bearing as yet unknown mutations are shown in table 3.

Discussion

FREQUENCY OF CF IN IRELAND AND VITAL STATISTICS

We have identified 1014 Irish CF patients on record by the end of December 1994. Care

Table 2 Haplotypes associated with the most common CF mutations in Ireland, as well as with Irish CF chromosomes with unidentified mutations and non-CF chromosomes

Mutation	Nm/Ncf*	%	X/K†	M‡	Microsatellite§	No chrs‖
ΔF508	367/506	72·5	A	1	See table 3	2
			B	2		116
			C	–		0
			D	2		2
G551D	35/506	6·9	B	2	16-7-17	20
R117H	10/506	2·0	C	1	16-30-13	5
			C	1	16-31-13	1
G542X	5/506	1·0	B	2	17-32-13	2
621+1G-T	4/506	0·8	B	2	21-7-17	2
			B	2	21-31-13	2
R560T	4/506	0·8	D	2	16-7-17	1
			D	2	16-31-17	1
1717−1G-A	3/506	0·6	C	1	16-32-13	2
			A	2	17-32-13	1
N1303K	2/506	0·4	B	2	23-29-13	2
3659delC	2/506	0·4	C	1	16-35-13	2
ΔI507	2/506	0·4	ND		ND	
R352Q	1/506	0·2	C	1	16-31-13	1
R553X	0/506	0·0	ND		ND	
1078delT	0/506	0·0	ND		ND	
Total identified	435/506	85·6				
Unidentified	71/506	14·4	A	1	See table 3	3
			A	2		5
			B	2		17
			C	1		5
			D	2		8
Normal			A	1	See table 3	16
			A	2		1
			B	2		3
			C	1		13
			C	2		1
			D	2		2

* Nm = number of chromosomes bearing the mutation. Ncf = number of total CF chromosomes screened (see text for methods). † A = allele 1 for KM-19 and allele 1 for XV-2c, B = alleles 1 and 2, C = alleles 2 and 1, D = alleles 2 and 2, respectively. ‡ M refers to linked marker Mp6-dp, which has also two alleles, 1 and 2. § Triplets of figures represent numbers of dinucleotide repeats found at loci IVS8CA, IVS17bTA, and IVS17bCA respectively. ‖ No of chromosomes haplotyped for all markers mentioned. ND = not done.

Table 3 Microsatellite haplotypes found in Irish normal and CF chromosomes

Normal chromosomes			*Non-ΔF508 chromosomes‡*			*ΔF508 chromosomes*		
*Haplotype**	*No*	*%*	*Haplotype**	*No*	*%*	*Haplotype**	*No*	*%*
16-30-13	20	18·3	16-7-17	17	34	23-31-13	72	48
16-31-13	16	14·7	16-31-13	5	10	17-32-13	34	23
16-32-13	10	9·2	17-7-17	3	6	17-31-13	12	8
16-7-17	8	7·3	16-30-13	4	8	23-32-13	12	8
16-46-13	4	3·7	16-29-13	2	4	22-31-13	5	3
16-34-13	4	3·7	23-37-13	2	4	23-30-13	3	2
17-30-13	3	2·7	17-37-11	2	4	16-31-13	3	2
17-7-17	3	2·7	23-31-13	2	4	16-32-14	2	1·6
16-29-13	3	2·7	24-7-17	1	2	17-34-13	1	0·6
16-25-13	2	1·8	17-29-14	1	2	17-41-13	1	0·6
16-32-13	2	1·8	15-7-17	1	2	16-31-14	1	0·6
16-45-13	2	1·8	23-36-13	1	2	17-7-17	1	0·6
23-22-17	2	1·8	17-32-13	3	6	21-7-17	1	0·6
16-35-13	2	1·8	16-7-13	2	4	23-31-17	1	0·6
16-51-13	2	1·8	16-32-13	1	2	18-32-13	1	0·6
Observed only once†	26		23-32-13	2	4			
			16-25-13	1	2			
Total	109			50			150	

* See legend for table 2.
† The following haplotypes were found only once in normal chromosomes: 16-36-13, 16-7-13, 16-50-13, 16-24-13, 16-33-13, 16-38-13, 16-49-13, 16-31-14, 16-29-17, 17-49-11, 17-50-11, 17-36-13, 17-29-14, 17-22-13, 17-7-11, 17-7-19, 18-7-13, 18-32-13, 18-35-13, 18-36-13, 21-32-13, 21-34-13, 23-30-13, 23-32-13, 23-33-13, and 24-39-13 (0·9% each).
‡ These do not include the mutations shown in table 2.

was taken to ensure that this was as close as possible to complete ascertainment by cross checking several sources. Average incidence at birth was estimated at 1/1461, which is comparable to the incidence reported for Brittany[22] and among the highest in the world. In their one year survey of paediatric hospital records, O'Reilly *et al*[1] reported an incidence of 1/1800 children under 1 year of age, but this reflects only 64% of all cases, since we have found that 36% CF patients are diagnosed after that age. Twenty percent of Irish CF patients are aged 20 years or older, but survival rate still falls drastically after that age.

Table 1 shows the current age distribution of CF patients in Ireland, which is comparable to the distribution in the US in 1990,[21] except for the relatively smaller proportion of patients aged 5 years or less in Ireland. This probably reflects the recent sudden decline in the number of Irish births, which dropped from a total of 74 080 in 1980 to 52 947 a decade later.[11] In the US, the proportion of adult patients had increased fourfold between 1969 and 1990.[21] A similar increase cannot be documented in Ireland for lack of retrospective data, but the increasing age of the Irish CF population required the opening of the first Irish adult CF clinic in 1977,[2] now attended regularly by about 200 patients. The upward shift in age distribution reflects improved care, and perhaps also improved rates of diagnosis. Patients bearing "mild" mutations, such as R117H, are frequently diagnosed late compared to those homozygous for ΔF508. They have a milder course of the disease[23] and can be confidently classified as CF patients after genotyping, thus probably contributing to the increasing age of the patient population. Two unrelated compound heterozygotes ΔF508/1717-1G-A diagnosed at 12 and 29 years of age are unusual because this has been found in association with a severe phenotype in other patients.[23] Both of these patients carry the 1717-1G-A on a different haplotype to that of the third patient of this genotype diagnosed at 1 month (table 2); it is quite possible that this haplotype difference coincides with another alteration within one of their CF genes which could modify the effect of the genotype, as observed by Dork *et al*[24] in a patient bearing three mutations on the CFTR gene.

Infertility in female CF patients is considered to be greater than 10%.[25] About 98% of males are infertile, mostly because of bilateral congenital absence of the vas deferens (CBAVD).[26] The observation of genotype ΔI507/R117H in a CF patient showing a mild form of the disease and who has fathered a child is interesting. Mutation ΔI507 is assumed to have the same clinical effects as the more common ΔF508.[27] Mutation R117H is usually found in mild disease, and has been reported in 10% of male compound heterozygotes expressing CBAVD (reviewed by Pignatti[28]), most frequently in association with ΔF508. The genotype ΔF508/R117H has also been found in an asymptomatic woman, and recent evidence suggests that the variable clinical manifestations found in compound heterozygotes bearing mutation R117H may be determined by "genetic background".[29] The case presented here adds to this phenotypic variability, since he is both fertile and pancreatic sufficient. However, unilateral absence of the vas deferens has not been excluded, nor has "genetic background", as referred to by Kiesewetter *et al*,[29] been investigated in this patient as yet.

FREQUENCY OF MUTATIONS ON THE CFTR GENE AND ASSOCIATED HAPLOTYPES

Table 2 shows the most common mutations in Ireland. Mutation ΔF508 has been reported in >70% of CF chromosomes from all laboratories in the British Isles,[4] except for Northern Ireland. Another two or three mutations are found in an additional 10% of CF chromosomes in all laboratories from the British Isles.[4] Mutation 1078delT appears to be relatively frequent exclusively in Brittany,[30] but was absent among 40 Irish CF non-ΔF508 chromosomes, and is also very infrequent in England,[4] suggesting that it might not be a marker common to Celtic populations as proposed by Audrezet *et al*.[30] Instead, the distribution of mutation G551D

appears to follow more closely the area of long term Celtic settlements,[31] and in view of the identical haplotype in all G551D chromosomes, we have suggested that all cases may be identical by descent.[10] Mutation R117H, found initially in a US patient of Anglo-Irish ancestry,[32] occurs with the highest frequency in Ireland, and with much lower frequency or not at all elsewhere.[4]

The haplotype associations shown in table 2 could be useful for counselling in some cases, for instance in families where DNA from the affected child is unobtainable,[33] or where a direct test for the less common mutations is not available. As has been observed in other populations,[34] there exists a strong association between the B (X/K:1/2) haplotype and the CF chromosome, even for those with uncharacterised mutations. This mutation occurs with low frequency in the sample of normal chromosomes. Microsatellite haplotypes have been studied in only 42 Irish chromosomes bearing the less common mutations (table 2), but the consistency of their association with certain mutations is already apparent. For instance, table 2 shows that mutations G542X, 621+1G−T, N1303K, 3659delC, R560T, and R117H are associated with the same haplotypes, or with haplotypes probably derived from these by slippage of the DNA polymerase, as has been seen in patients from other European populations.[7–10] Since those haplotypes are relatively rare in normal chromosomes (table 3), the constancy of the association has been explained as suggesting identity by descent of all chromosomes bearing the same mutations.[9] Haplotype 16-7-17 is found in CF non-ΔF508 chromosomes more frequently than would be expected from its frequency in normal chromosomes ($p<0{\cdot}01$, table 3), suggesting perhaps that another fairly common CF mutation may be associated with this haplotype in Ireland.

As the patient population benefits from longer and better quality of life, and the bases for new treatments are found, accessibility and concentration of clinical and genetic data are more necessary, and this would appear to justify the additional effort required to complete the database.

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