P67L: a cystic fibrosis allele with mild effects found at high frequency in the Scottish population

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Abstract

Only three mutant cystic fibrosis (CF) alleles have to date been established as conferring a dominant mild effect on affected subjects who are compound heterozygotes. We now add a fourth, P67L, which occurs on about 1.4% of Scottish CF chromosomes. Among 13 patients (12 unrelated) with this allele, the average age at diagnosis was 22.5±11.3 years. None of the cases had consistently raised sweat chloride concentrations, the average value being 57±9 mmol/l; 77% of the patients were pancreatic sufficient. When compared to three other established mild CF alleles, R117H, A455E, and 3849+10kb C-T, a compound heterozygote for P67L has minimal disease and clinical suspicions are unlikely to be confirmed other than by DNA typing.

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Since the cloning of the cystic fibrosis (CF) gene in 1989, a small number of mutant alleles have been found which can be described as having a "dominant mild" effect; the phenotypic effect is mild whatever the nature of the mutant allele on the other chromosome. In a majority of cases these alleles have been so rare that only one or two affected cases have been identified. Kerem and Kerem¹ suggested that before a genotype-phenotype correlation could be regarded as established, there should be at least 10 unrelated cases of CF patients bearing that allele. On this basis the only accepted mild CF alleles would be R117H,² A455E,³ and 3849+10kb C-T.⁴ The criteria used by Kerem and Kerem¹ to define a mild CF phenotype were: diagnosis at a later age, older at the time of the study, lower sweat chloride levels (some with normal or borderline values), better nutritional status, and (usually) pancreatic sufficiency.

The mutation P67L was first described by Hamosh *et al.*⁶ In the course of a survey of Scottish CF patients we discovered that it occurred more frequently than had been expected. Furthermore, the presentation was in almost all cases atypical. In this report we summarise our experience with 13 CF patients (12 unrelated) who are compound heterozygotes for P67L and other mutant alleles and review the evidence for a dominant mild phenotype.

Materials and methods

PATIENTS

Patients DO and AL were referred as part of investigation into their infertility. In neither case had cystic fibrosis been suspected until quite late in life. At the ages of 36 and 34, respectively, DO and AL were found to have bilateral absence of the vas deferens. However, DO had attended the ENT Department for sinus problems and had had nasal polyps removed when in his teens.

EM presented at hospital aged 9 with cough and breathlessness. Sweat tests were equivocal and he was diagnosed as having asthma. He had a number of chest infections with wheeze over the next few years. At 15, he was thought to have pulmonary aspergillosis. At 30, finger clubbing was seen and a chest x ray suggested bronchiectasis. Sputum culture grew *Haemophilus influenzae*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. At 32, he developed mild malabsorption and was then investigated for CF.

AM was diagnosed as having ulcerative colitis after 8 to 9 years of persistent diarrhoea. At the age of 27, the response of her diarrhoea to pancreatic supplements, a borderline sweat test, productive cough and streaky shadowing on chest x ray, and culture of *Staphylococcus aureus* and *Haemophilus influenzae* in sputum led to a clinical diagnosis of CF. However, as both her children had persistent respiratory symptoms, it was later suspected that both AM and her partner might instead be heterozygotes. This was not resolved until the molecular diagnosis.

In childhood, ED had symptoms suggestive of irritable bowel syndrome. Ciliary studies identified abnormally short cilia with a disorganised and focally asynchronous beat. Later she had recurrent chest infections and at the age of 32 chest x ray showed evidence of bronchiectasis, while sputum cultures showed *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Haemophilus influenzae*. She is currently pregnant.

The diagnosis in JM had been made at the age of 19 on the basis of a persisting productive cough, occasional haemoptysis, and breathlessness. There was also apparently a positive sweat test, though the date and the records have been lost.

MA was referred to the adult CF clinic at the age of 21 with an unconfirmed diagnosis of CF. Although reported as having recurrent chest illnesses as a child, he has at present few signs

Table 1 Characteristics of P67L patients

Patient	Sex	Age at 3/97	Age at diagnosis of CF	Original primary diagnosis	Genotype P67L/	Pancreatic status*
DO	M	39	38	Male infertility	ΔF508	PS
EM	M	35	33	Asthma	ΔF508	PS
AL	M	34	34	Male infertility	ΔF508	PS
AM	F	34	32	Ulcerative colitis	ΔF508	PI
ED	F	33	33	Primary ciliary dyskinesia	ΔF508	PS
JM	M	30	19	Persistent cough, possible CF	ΔF508	PS
MA	M	27	27	Probable CF	ΔF508	PI
LB	F	21	19	Asthma	1717-1G-A	PS
JC	M	20	18	Recurrent chest infections	G542X	PI
KB	F	19	16	Respiratory disease	ΔF508	PS
BH	F	15	13	Asthma	Q1291H	PS
SH	M	12	9	Possible CF	Q1291H	PS
AT	F	1	1	Probable CF	ΔF508	PS

*PI, pancreatic insufficient; PS, pancreatic sufficient.

of the disorder and has never been admitted to hospital.

LB's presentation was suggestive of asthma but she had poor initial response to bronchodilator therapy and inhaled steroids. At the age of 19 she was diagnosed as having CF after a positive sweat test. Subsequently, her symptoms improved on regular steroid therapy.

JC had recurrent chest infections and an equivocal sweat test. When CF was diagnosed on the basis of P67L/G542X compound heterozygosity, it was pointed out that his younger brother (but not his older brother) had identical symptoms. His younger brother is a P67L heterozygote, his older brother a G542X heterozygote.

KB had a history of chest infections but a normal sweat test at the age of 12. At 16 the presence of significant respiratory disease and nasal polyps led to reinvestigation of the diagnosis by molecular methods.

BH and SH are sibs. SH had an abnormal sweat test at the age of 3, but thereafter doubts arose about the diagnosis of CF. She has a complex chromosomal rearrangement involving chromosomes 1, 13, and 16 and moderately severe learning difficulties. Her older brother, BH, is perfectly normal in all respects except for a tendency to asthma.

Table 2 Sweat test data on P67L patients

Patient	Age when tested	Sodium (mmol/l)	Chloride (mmol/l)	Interpretation*
DO	Never			
EM	14		62	Equivocal
AL	14		50	Equivocal
AM	Never			
AM	26	86	77	Abnormal
AM	26	70	63	Equivocal
AM	26	67	60	Equivocal
ED	33	85	67	Equivocal
ED	33	81	58	Equivocal
JM	?	Records lost		Abnormal
MA	19	97	69	Equivocal
LB	19	Screening chloride electrode		Abnormal
JC	8	53	55	Equivocal
JC	14	50	56	Equivocal
JC	14	43	54	Equivocal
KB	12	49	37	Normal
KB	15	67	63	Equivocal
BH	8	99	80	Abnormal
BH	14	49	38	Normal
SH	3	82	75	Abnormal
SH	3	61	61	Equivocal
SH	6	60	46	Normal
SH	11	72	65	Equivocal
AT	1	83	87	Abnormal
AT	1	56	58	Equivocal
AT	1	53	54	Equivocal

*Conclusion reported by testing laboratory

AT was the product of IVF from a P67L sperm donor. She had a collapsed right upper lobe at presentation aged 1 year. Her first sweat test was abnormal and the next two equivocal. Her early diagnosis was the result of P67L screening being introduced for cases where a diagnosis of CF was possible.

METHODS

DNA analyses

Samples referred from patients where there was a possible diagnosis of CF were tested for the presence of up to 26 mutant alleles: ΔF508, G551D, G542X, R117H, ΔI507, V520F, R553X, 621+1G-T, A455E, N1303K,⁷ R560T,⁸ Q493X,⁸ C524X,⁹ Q1291H,⁹ G85E,¹⁰ 1717-1G-A,¹¹ D110H,¹² G458V,¹³ W1282X,¹⁴ R1283M,¹⁵ 4005+1G-A,¹⁶ G1244E,¹⁷ D1270N,¹⁸ S1255X,¹⁹ 1898+1G-A,²⁰ and P67L.⁶ The first 10 of the above were tested in a single tube multiplex system⁷ and the remainder by the referenced methods.⁸⁻²⁰ The three locus IVS8CA-IVS17bTA-IVS17bCA haplotype was scored by the method of Hughes *et al.*²¹

Sweat testing

For six of the patients (AM, MA, KB, BH, SH, AT) sweat testing was carried out in Edinburgh as described previously.²² In two cases (DO, AL) sweat tests were never performed since there has not been a clinical indication. The remaining five cases were tested in other centres.

Pancreatic status

Pancreatic status of the three youngest patients was established by stool trypsin measurement.²³ In the other cases, assessment was made on the basis of a normal growth pattern, normal stools, and absence of response to pancreatic enzymes.

Results

Diagnostic characteristics of the 13 P67L patients are shown in table 1. Most were compound heterozygotes for ΔF508. Ten of the 13 were pancreatic sufficient. If patient AT is ignored (because she was detected after the introduction of P67L screening for "possible" CF cases), the median age of the other 12 is 28.5 years and the median age of diagnosis 23 years. However, this includes scoring JM's

Table 3 Comparison of four dominant mild alleles

Allele	No of patients	Age, y (SD)	Age at diagnosis, y (SD)	PS %	Sweat chloride, mmol/l (SD)	Ref
R117H	23	23.5 (9.6)	10.2 (10.5)	87	80 (18)	2
A455E	33	23	15.0 (10.6)	79	NG*	3
3849+10kb	15	20.5 (9.9)	12.5 (8.8)	67	62 (17)	4
P67L	13	24.6 (11.1)	22.5 (11.3)	77	57 (9)	

*NG, not given

diagnosis as having occurred at the age of 19 on the basis of a now lost positive sweat test. The average age and the average age of diagnosis of the whole set of 13 was 24.6 (SD 11.1) and 22.5 (SD 11.3) years, respectively.

Sweat test data are shown in table 2. Six of the cases were tested at the Royal Hospital for Sick Children, Edinburgh, which cites a normal sweat chloride for any age as <50 mmol/l and between 50 and 70 as "equivocal".²² Owing to increasing values with advancing age, sweat sodium measurement is less definitive and some normal adults may have values >70 mmol/l.²² In table 2, "interpretation" means the conclusion reported by the laboratory carrying out the testing. The two infertility patients have never been tested. In all other cases the data were regarded as too equivocal to support a diagnosis of CF. Taking only the most recent determination for each patient, the average sweat chloride concentration was 57±9 mmol/l (n=9) and the average sweat sodium 66.1±17.8 mmol/l (n=8).

In four of the 13 cases the intragenic IVS8CA-IVS17bTA-IVS17bCA haplotype associated with the P67L mutation was 16-7-17, as previously found.²¹ In one case (LB) the patient was homozygous for this haplotype and in another three (JC, BH, SH) parents were available for direct testing. In the remaining nine cases the 16-7-17 haplotype could be inferred to be associated with P67L.

If the two cases where the first presentation was infertility are ignored, P67L was found on 10 independent CF chromosomes of 730 scored (1.4%).

Discussion

The P67L allele was first reported by Hamosh *et al*⁶ as a mutation found in a pancreatic sufficient patient. However, only two further cases have been subsequently reported, both without clinical data.²¹ We were therefore surprised at its relatively high frequency. Our estimate of 1.4% of CF chromosomes makes it the sixth most common mutation (after ΔF508, G551D, G542X, R117H, and 1717-1G-A) in Scotland. Almost certainly, given the atypical nature of most presentations, this is a considerable underestimate. For example, we find another mild allele, R117H, occurring on 2.4% of CF chromosomes from homozygotes and on 6.9% of CF chromosomes from unselected heterozygotes. It is interesting that all 13 P67L mutations were on the same genetic background, 16-7-17, which occurs on only about 11% of normal chromosomes.²¹

For many of the cases, with the exception of the two male infertility patients, there was some suspicion of CF at earlier ages. However, the

diagnosis could not be confirmed by sweat test for, as table 2 shows, none of the patients had consistent chloride values in the pathological range. Furthermore, only three of the 13 were pancreatic insufficient. This, together with the variability in presenting symptoms, means that it is no surprise that the median age of diagnosis was 19 years (average 22.5), against a 1984 to 1990 median age of diagnosis of children with CF in the Edinburgh area of 5 months.²²

Our claim that P67L confers a "dominant mild" phenotype rests on mild disease being found in patients who were compound heterozygotes for other known severe alleles (ΔF508, G542X, and 1717-1G-A). A possible exception to this are the sibs BH and SH who had Q1291H on the other allele. To our knowledge this allele has only been found in three cystic fibrosis patients, the two here (one of whom was also the subject of the first Q1291H report⁹) and one other. The third case, also originating in Scotland, was from a child who died in hospital aged 3 years, having been an emergency admission with a 10 day history of upper respiratory tract infection. Although the other CF allele is not known in this case, the existence of such severity argues against Q1291H conferring the dominant mild effect in P67L/Q1291H compound heterozygotes.

In a recent survey by Kerem and Kerem,¹ only three CF alleles, R117H, A455E, and 3849+10kb C-T, were regarded as unequivocally conferring a dominant mild effect. Two others, G85E and R334W, were seen as "substantially variable". Table 3 compares the characteristics of P67L with the three accepted as being mild. In terms of age, age at diagnosis, probability of pancreatic sufficiency, and normality of sweat chloride levels, P67L compound heterozygotes are probably the least seriously affected of any CF case where the molecular pathology has been established.

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