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Intron-8 polythymidine sequence in Australasian individuals with CF mutations R117H and R117C

R.J.H. Massie*, N. Poplawski#, B. Wilcken¶, J. Goldblatt+, C. Byrnes§, C. Robertson*

Intron-8 polythymidine sequence in Australasian individuals with CF mutations R117H and R117C. R.J.H. Massie, N. Poplawski, B. Wilcken, J. Goldblatt, C. Byrnes, C. Robertson. ©ERS Journals Ltd 2001.

ABSTRACT: Compound heterozygotes for a severe cystic fibrosis transmembrane conductance regulator (CFTR) mutation and the R117H or R117C mutation (R117H/C) have clinical presentations that vary from classic cystic fibrosis (CF) to an incidental genetic finding.

The aim of this study was to assess the influence of the intron-8 polythymidine sequence (IVS8) on the relationship between genotype and phenotype of individuals with R117H/C.

All individuals with R117H/C known to CF clinics in Australia and New Zealand were retrospectively studied by collecting information on genotype, age, pancreatic status, sweat electrolytes, sputum microbiology and pulmonary function.

Forty-one individuals (39 with R117H and two with R117C), 16 on an IVS8-5T background and 25 on an IVS8-7T background were identified. Twelve individuals presented clinically, four were siblings of known R117H/C compound heterozygotes and 25 were detected by newborn screening. Eleven of 14 of the IVS8-5T group (78%) with sweat chloride results available had sweat $Cl > 60$ mmol·L^{-1} compared to 5 (20%) of the R117H/7T group (Chi-squared = 10.4, p = 0.001). Two were pancreatic insufficient, both IVS8-5T. Two IVS8-5T individuals have recently died (aged 43 and 19) and of the 14 surviving IVS8-5T group, 11 (79%) are symptomatic compared to eight (32%) of the IVS8-7T individuals (Chi-squared = 6.1, p = 0.01).

In conclusion, most individuals with R117H/C on a IVS8-5T background have an elevated sweat chloride and clinical cystic fibrosis, which in some cases is severe. Most individuals with R117H/C on an IVS8-7T background do not have clinical cystic fibrosis but should be followed for the development of clinical disease.

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*Dept of Respiratory Medicine, Royal Children's Hospital, Melbourne, Australia, #Dept of Chemical Pathology, Women's and Children's Hospital, North Adelaide, Australia, ¶Royal Alexandra Hospital for Children, Sydney, Australia, +Princess Margaret Hospital for Children, Perth, Australia and §Starship Children's Hospital, Auckland, New Zealand.

Correspondence: R.J.H. Massie
Dept of Respiratory Medicine
Royal Children's Hospital
Melbourne 3052
Australia
Fax: 61 393491289

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The correlation between genotype and phenotype in cystic fibrosis (CF) is not always clear [1]. In general, individuals who are compound heterozygotes for a severe cystic fibrosis transmembrane conductance regulator (CFTR) mutation and R117H or R117C (R117H/C) have milder disease. However, the phenotype can range from classical CF, atypical CF-like lung disease, to isolated congenital absence of the vas deferens (CAVD), or be discovered incidentally, at a time when the individual is asymptomatic [1, 2]. R117H/C are class IV mutations associated with the production of a CFTR protein which has altered channel properties [3, 4]. Chloride transport through R117H/C CFTR is reduced and this is thought to explain the milder phenotype [3, 4].

The variable presentation amongst R117H/C compound heterozygotes may be explained, in part, by the efficiency of exon-9 splicing. Exon-9 splicing is influenced by the polythymidine sequence of intron-8 (IVS8) which immediately precedes the splice acceptor site [5]. The polythymidine tract is polymorphic with sequences of 5, 7 or 9 thymidines (T) and exon-9 splicing is inversely proportional to thymidine sequence length [6, 7]. CFTR missing exon-9 (exon-9 CFTR) is not functional as it lacks the first nucleotide binding fold. The effects of this splicing variant are in addition to those of mutations at other sites.

The variable presentation of individuals with R117H/C makes it difficult to be certain of the clinical outcome. However, the widespread availability of CFTR gene mutation testing, in particular through newborn screening programmes and antenatal testing, has created the need to predict the prognosis of individuals who are compound heterozygotes for a severe CFTR mutation and R117H/C who may be asymptomatic at the time of testing. The North American Cystic Fibrosis Foundation Consenus Panel has recently proposed criteria for the diagnosis of CF [8]. The criteria includes the presence of two disease producing CFTR mutations of which R117H/C are only considered disease producing mutations when *in cis* with IVS8-5T [8]. Despite this, there are reports of individuals with CF-like conditions who have R117H/C with the IVS8-7T allele [5, 9, 10] and there

is no definitive information as to strength of the relationship between R117H/C, the IVS8 polythymidine sequence and the clinical outcome.

The aim of this study was to assess the relationship between genotype and phenotype of individuals known to have the R117H/C mutation and the influence of the IVS8 polythymidine sequence.

Subjects and methods

The directors of all Australian and New Zealand (Australasian) CF clinics and Clinical Genetics Services involved with CF mutation analysis were contacted by letter. Information was requested regarding all individuals, alive or deceased, who had been seen in their clinic and who were known to have the R117H or R117C mutation. R117H and R117C have been considered to be functionally equivalent [11] and have been combined in the analysis, referring to R117H and R117C collectively as R117H/C. The information recorded included the mutation at both alleles, IVS8 sequence of both alleles, symptoms leading to the detection of the genotype and where available, the age, sex, sweat electrolyte concentration, pancreatic status, sputum culture, pulmonary function test results (forced expiratory volume in one second (FEV_1)) and clinical status of the patient (pulmonary, gastrointestinal, liver, absence of the vas deferens or other symptoms).

Individuals were known to CF clinic directors and Clinical Genetics Services on the basis of either a clinical presentation (symptoms of CF or a sibling of a patient compound heterozygote for R117H/C) or identification through newborn screening. Newborn screening for CF is routine in all states of Australia (except Western Australia) and in New Zealand. The year that newborn screening for CF commenced varied across different centres, with the first centre (New South Wales) starting in 1981 and Western Australia not screening at the time of this study. All centres that screen currently use neonatal immunoreactive trypsinogen (IRT) as the primary screen followed by ΔF508 mutation analysis in those with an elevated (>99th percentile) IRT. One Australian centre (South Australia) includes the mutations G551D, G452X, ΔI507, R553X and R117H as part of routine screening of infants with an elevated IRT [12]. All individuals with a positive result on newborn screen (either one or two mutations) are referred for sweat testing. Infants with a positive (>60 mmol·L^{-1}) or borderline (40–60 mmol·L^{-1}) sweat chloride and in whom there is an unidentified mutation are referred for an extended mutation analysis which includes: ΔF508, R117H, G551D, A455E, G542X, N1303K, W1282X, 1717-1, R560T, R347P, R334W, R1162X, S549N, 621+1, 3849+10C>T, and the IVS8 polythymidine sequence. One Australian centre (New South Wales) performed the extended mutation analysis on all ΔF508 heterozygotes with a negative (<40 mmol·L^{-1}) sweat chloride between 1995–1996 as part of a study to evaluate newborn screening [13].

The method used for mutation detection varies by centre and with the mutation screened and includes allele specific oligonucleotide hybridization (ASO) [14], restriction enzyme digests, multiplex amplification refractory mutation systems (ARMS) [15] and heteroduplex analysis.

The IVS8 polythymidine sequence was detected by ASO [5] (New South Wales, South Australia), nested polymerase chain reaction (PCR) with product siz ing by gel electrophoresis [16] (Western Australia, Victoria) or minisequencing (New Zealand). R117H was detected by ARMS (Western Australia, New South Wales, Victoria, Tasmania, New Zealand) or ASO (South Australia) and R117C was detected using a restriction enzyme digest (Western Australia, New South Wales, Victoria, Tasmania), ASO (South Australia) or ARMS (New Zealand).

For all individuals, sweat electrolytes were performed by pilocarpine iontophoresis with collection by either Gibson and Cooke pad technique [17] or Wescor Macroduct. Sweat $Cl > 60$ mmol·L^{-1} was considered diagnostic for CF, 40–60 mmol·L^{-1} borderline and <40 mmol·L^{-1} in the normal range [17]. Some individuals had more than one sweat test and the highest value has been used for analysis. Abnormal pancreatic status was confirmed by the presence of fat globules in the stool, where possible by 3-day faecal fat collection and in some cases by formal pancreatic stimulation testing. Microbiological assessment of the sputum was made from individuals who have expectorated sputum. Individuals were considered to have CF related respiratory symptoms if they had a productive cough, recurrent bronchitis or bronchiectasis but not asthma alone. Pulmonary function studies were performed in respiratory function laboratories attached to each of the CF clinics on all individuals >6 yrs. Individuals who presented to infertility clinics with isolated CAVD but were not receiving follow-up by a respiratory medicine specialist or at a CF clinic were excluded from the study.

Results

Genotype

Forty-one individuals with R117H/C (39 R117H and two R117C) being followed at one of the CF clinics in Australia and New Zealand were identified. The genotype and clinical information for each is presented in table 1. Thirty-four of the 39 individuals were compound heterozygotes for R117H/C and ΔF508. Two of the individuals had a second severe mutation (G542X/R117H and G551D/R117H), four had an unknown second mutation and one patient was homozygous for R117H.

Sixteen individuals had R117H on an IVS8-5T background and 25 on an IVS8-7T background. The phase of each chromosome was unknown, preventing identification of the direct association between R117H and the IVS8 polythymidine sequence. However, previous studies have indicated that where present, IVS8-9T occurs *in cis* with ΔF508 [5] and that R117H is the only mutation that occurs *in cis* with IVS8-5T [18].

Table 1. – Clinical information on R117H and R117C subjects

Subject	Genotype	IVS8	Current Age yrs	Sex	Sweat Cl mmol·L^{-1}	Pancreatic status	Presentation	Sputum culture	FEV1 % pred	Other
1	ΔF508/R117H	9T/5T	6	M	58, 40	PS	Clinical	PA, SA	74	Daily cough
2	ΔF508/R117H	9T/5T	8	F	41, 84	PS	Clinical	SA	84	Daily cough
3	ΔF508/R117H	9T/5T	14	F	73	PI	Clinical	SA	99	Daily cough, CBD obstruction
4	ΔF508/R117H	9T/5T	5	M	53	PS	NBS	Clear	Too young	Daily cough
5	ΔF508/R117H	9T/5T	4	F	42, 46	PS	NBS	No sputum	Too young	Asymptomatic
6	ΔF508/R117H	9T/5T	Died (43)	F	91	Unclear	Clinical	Alcalogenes		Died
7	ΔF508/R117H	9T/5T	49	M	98	PS	Clinical	PA	40	Daily cough
8	ΔF508/R117H	9T/5T	17	M	79, 82, 84	PS	Clinical	SA, HI	97	Atrophic vas
9	ΔF508/R117H	9T/5T	4	F	Insufficient	PS	NBS	SA, SPn	Too young	Occasional cough
10	ΔF508/R117H	9T/5T	Died (19)	M	85	PS	Clinical	PA		Died
11	ΔF508/R117H	9T/5T	1	M	118	PS	NBS	SA, HI	Too young	Asymptomatic
12	ΔF508/R117H	9T/5T	14	M	62	PS	Clinical	SA	86	Bronchiectasis
13	Unknown/R117H	7T/5T	38	F	116	PS	Clinical	PA, HI, SA	64	Daily cough, 6 monthly *i.v.* AB
14	Unknown/R117H	9T/5T	11	M	71, 40, 79	PS	NBS	Clear	88	Occasional cough
15	Unknown/R117H	9T/5T	7	F	61, 58, 79	PS	NBS	SA	111	Occasional cough
16	G542X/R117H	9T/5T	44	M	No record	PI	Clinical	HI	111	Clear chest
17	ΔF508/R117H	9T/7T	2	F	27	PS	NBS	No sputum	Too young	Asymptomatic
18	ΔF508/R117H	9T/7T	2	F	21	PS	NBS	No sputum	Too young	Asymptomatic
19	ΔF508/R117H	9T/7T	3	M	32, 39	PS	NBS	No sputum	Too young	Asymptomatic
20	ΔF508/R117H	9T/7T	3	M	20, 22	PS	NBS	No sputum	Too young	Asymptomatic
21	ΔF508/R117H	9T/7T	3	F	21, 45, 22	PS	NBS	No sputum	Too young	Asymptomatic
22	ΔF508/R117H	9T/7T	1	M	52, 64	PS	NBS	Clear	Too young	Asymptomatic
23	ΔF508/R117H	9T/7T	2	M	40, 34, 33	PS	NBS	No sputum	Too young	Asymptomatic
24	ΔF508/R117H	9T/7T	3	M	38, 37, 56	PS	NBS	No sputum	Too young	Asymptomatic
25	ΔF508/R117H	9T/7T	11	M	23	PS	Sibling	No sputum	62	Atrophic vas
26	ΔF508/R117H	9T/7T	14	M	23	PS	Sibling	No sputum	86	Asymptomatic
27	ΔF508/R117H	9T/7T	6	M	42, 57,31	PS	NBS	No sputum	84	Recurrent bronchitis
28	ΔF508/R117H	9T/7T	8	M	37, 40	PS	NBS	No sputum	92	Asthma
29	ΔF508/R117H	9T/7T	1	F	24, 34, 40	PS	NBS	No sputum	Too young	Persistent cough
30	ΔF508/R117H	9T/7T	2	F	47	PS	NBS	No sputum	Too young	Asymptomatic
31	ΔF508/R117H	9T/7T	3	M	29, 60	PS	NBS	No sputum	Too young	Asymptomatic
32	ΔF508/R117H	9T/7T	6	F	23, 37	PS	NBS	No sputum	133	Asthma
33	ΔF508/R117H	9T/7T	2	F	24, 23	PS	NBS	No sputum	Too young	Asymptomatic
34	ΔF508/R117H	9T/7T	6	M	25, 34	PS	NBS	No sputum	105	Asymptomatic
35	ΔF508/R117H	9T/7T	27	F	57	PS	Clinical	SA, HI	63	Adult diagnosis, Bronchiectasis
36	ΔF508/R117H	9T/7T	34	F	53	PS	Sibling	No sputum	82	Asymptomatic
37	G551D/R117H	7T/7T	6	M	70, 58	PS	NBS	S Pn, B cat, HI	Too young	Daily cough
38	Unknown/R117H	9T/7T	31	M	95, 102	PS	Clinical	PA, SA	98	Daily Cough, Atrophic vas
39	ΔF508/R117C	9T/7T	5	M	43, 48, 85	Unclear	NBS	No sputum	123	Recurrent cough, Occasional fatty stools
40	ΔF508/R117C	9T/7T	10	M	57, 50	Unclear	Sibling	No sputum	106	Occasional chest infection
41	R117H/R117H	7T/7T	1	F	23	PS	NBS	No sputum	Too young	Asymptomatic

IVS8: intron-8 polythymidine sequence; FEV1: forced expiratory volume in one second; M: male; F: female; PS: pancreatic sufficient; PI: pancreatic insufficient; PA: *Pseudomonas aeruginosa*; SA: *Staphylococcus aureus*; NBS: newborn screening; HI: *Heamophilus influenzae*; S Pn: *Streptococcus pneumonia*; B cat: *Brahmonella catarrhalis*; CBD: common bile duct; *i.v.*: intravenous; AB: antibiotics.

Presentation

Twenty-five of the 41 (61%) individuals were discovered through newborn screening, 12 (29%) because of respiratory symptoms and four (10%) were siblings of an R117H/C compound heterozygote (three had a younger sibling with a positive newborn screening test and one had a sibling presenting clinically).

Sweat electrolytes

Fourteen of the 16 individuals in the IVS8-5T group and all 25 of the IVS8-7T group had sweat test results available (table 2). The mean$\pm$SD sweat Cl for the IVS8-5T group was 80 ± 21 mmol·L^{-1} which was higher than the mean$\pm$sweat Cl for the IVS8-7T group, 46 ± 21 mmol·L^{-1} ($p<0.001$). The difference between the number of IVS8-5T and IVS8-7T individuals with a positive (>60 mmol·L^{-1}) sweat Cl was significant (Chi-squared = 10.4, $p=0.001$).

Pancreatic status

Thirty-six individuals (88%) were pancreatic sufficient (PS), two individuals (5%) were pancreatic insufficient (PI) and in three cases (7%) there was not accurate information to determine pancreatic status. Both PI individuals had R117H/C on an IVS8-5T background.

Microbiology

Sputum culture results were available for all of the IVS8-5T group but only five of the IVS8-7T group. Some individuals had more than one organism cultured (table 3). The reason for the discrepancy in obtaining sputum for microbiology between the groups relates to both the younger age and lack of pulmonary symptoms in the IVS8-7T group.

Pulmonary symptoms and function

There were two individuals who died in the last 2 yrs and who have been included in the study. Both had the ΔF508/R117H(9T/5T) genotype and died from suppurative lung disease at 19 and 43 yrs of age.

Of the 16 IVS8-5T individuals, 12 (75%) had CF related respiratory symptoms (including the two individuals that died) compared to seven (28%) of the 25 IVS8-7T individuals (Chi-squared = 8.7, $p=0.003$). There was a difference in the median age of the IVS8-5T and IVS8-7T groups (17.8 *versus* 7.7 yrs, Mann-Whitney $p=0.007$). However, age alone did not account for the difference in symptoms of the two groups. Using logistic regression, adjusting for age, the odds ratio of the difference in respiratory symptoms was 6.1 (95% confidence interval 1.36–26.9).

Of the surviving 39 individuals, 19 were old enough to perform reliable spirometry. The mean FEV1 (% predicted) of the surviving IVS8-5T individuals was not different from that of the IVS8-7T individuals (85% *versus* 95%, $p=0.4$).

Table 2. – Comparison of sweat chloride values between patients with the intron-8 polythymidine sequence (IVS8)-5T and IVS8-7T allele

IVS8	≥ 60 mmol·L^{-1}	40–59 mmol·L^{-1}	<40 mmol·L^{-1}	No sweat result	Total
IVS8-5T	11	3	0	2	16
IVS8-7T	5	10	10	0	25

Table 3. – Comparison of sputum microbiology isolates between patients with the IVS8-5T and IVS8-7T alleles

IVS8	PSA	SA	HI	Other	Clear	No Sputum
IVS8-5T	4	9	4	1	2	1
IVS8-7T	1	2	2	2	2	20

Some patients had more than one organism on sputum culture. PSA: *Pseudomonas aeruginosa*; SA: *Staphylococcus aureus*; HI: *Heamophilus influenzae*.

Other manifestations of cystic fibrosis

One subject has distal common bile duct obstruction. There are three individuals with CAVD identified. Two were diagnosed at herniotomy, although their genotype was already known from newborn screening (IVS8-5T and IVS8-7T) and one presented with infertility but was then found to have clinical features of CF and a sweat Cl >60 mmol·L^{-1}. None of the individuals had a history of sinusitis, nasal polyps or pancreatitis.

Discussion

This study provides evidence that the variable presentation of individuals with the R117H/C mutation is associated with differences in the IVS8. Most individuals with R117H/C and the IVS8-5T allele fulfil clinical and sweat test criteria for the diagnosis of CF while most individuals with R117H/C and the IVS8-7T allele do not [8]. Most of the IVS8-7T group were detected on newborn screening, while the majority on the IVS8-5T group were referred because of symptoms and as a consequence were older. However, adjusting for age there is still a difference in the presence of respiratory symptoms due to CF between the two groups.

In general, individuals who are compound heterozygotes for a severe mutation and R117H/C on an IVS8-5T background have pancreatic sufficient CF which is mild compared to individuals with two severe mutations [2]. However, the CF can be severe as proved by the two respiratory deaths in the present cohort and the additional two individuals who have pancreatic insufficiency. Amongst the IVS8-7T group were five individuals with sweat chloride

>60 mmol·L^{-1} and five others who were symptomatic with a borderline sweat chloride. Symptoms of established suppurative lung disease were present in some of the older IVS8-7T individuals, raising the possibility that significant lung disease may develop in more of the IVS8-7T group with time.

This study is similar to that of KIESEWETTER *et al.* [5] who studied 38 pancreatic sufficient CF individuals with the ΔF508/R117H genotype, of whom 31 had the IVS8-5T allele and seven the IVS8-7T allele. A further nine ΔF508/R117H individuals were studied, eight males had CAVD only and one female who was asymptomatic, and in each case the R117H was associated with the IVS8-7T allele. The difference between IVS8-5T and IVS8-7T distribution amongst symptomatic individuals with the ΔF508/R117H genotype was significant (Chi-squared = 15.2, $p<0.001$). This study recognized the need for IVS8 polythymidine analysis to explain the variability amongst individuals with the R117H mutation.

Other studies have found individuals with R117H on a IVS8-7T background to have pulmonary disease. DEAN *et al.* [9] reported a 56-yr-old female with R117H/IVS8-7T who had chronic bronchitis from the age of 10 yrs, complicated by allergic bronchopulmonary aspergillosis (ABPA). The subject was pancreatic sufficient and had a normal sweat test but clearly had respiratory features of atypical CF. Similarly, one patient in a series of sweat test negative ABPA individuals had R117H a IVS8-7T background and could be considered to have a mild form of CF [10].

The variability of clinical presentation of individuals with the R117H/C mutations is largely, but not entirely explained by the IVS8 polythymidine sequence. It has been suggested that the polymorphic (TG)m sequence which is immediately proximal to the polythymidine sequence may also affect exon-9 splicing by interacting with the splicing mechanism [19]. Furthermore, there is evidence that some CFTR polymorphisms, for example the M470V locus, previously thought to be a harmless polymorphism, may slow CFTR processing through the endoplasmic reticulum, so that less mature protein is released [19]. A more detailed study of the effects of these polymorphisms is warranted.

The importance of understanding the role of the IVS8 polythymidine sequence amongst individuals with the R117H mutation was highlighted in a recent report by CHMIEL *et al.* [20]. In this report an infant was diagnosed with CF on the basis of the detection of ΔF508 and R117H in cord blood but without IVS8 polythymidine sequencing being performed until much later. The infant was asymptomatic with two normal sweat chlorides (18 mmol·L^{-1} and 20 mmol·L^{-1}), a normal nasal potential difference and a normal bronchoalveolar lavage. The R117H was found to be on an IVS8-7T background and the diagnosis of CF was changed, but not without considerable emotional cost to the family. Clearly at this point in time this infant does not have CF according to Consensus Guidelines [8]. The early recognition of the IVS8-7T would have saved a great deal of anxiety and expense but there is still a possibility that the infant may develop a CF phenotype with time.

This problem is an example of the difficulties that confront centres that offer newborn screening and prenatal diagnosis. If R117H/C is detected then it is imperative that intron-8 polythymidine sequence analysis be performed to offer some guidance as to the likely phenotype. Most individuals with a severe cystic fibrosis mutation and R117H/C on the intron-8 polythymidine sequence-5T background will have an elevated sweat chlorine and clinical features of cystic fibrosis, which in some cases are severe and associated with an early death. Most individuals with R117H/C on the intron-8 polythymidine sequence-7T background do not have clinical features of cystic fibrosis although over half have elevated or borderline sweat chloride. Individuals with R117H/C on the intron-8 polythymidine sequence-7T background should be followed for the potential to develop clinical disease.

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References

1. Kerem E, Kerem B. Genotype-phenotype correlations in cystic fibrosis. *Pediatr Pulmonol* 1996; 22: 387–395.
2. The Cystic Fibrosis Genotype-Phenotype Consortium. Correlation between genotype and phenotype in patients with cystic fibrosis. *N Engl J Med* 1993; 329: 1308–1313.
3. Sheppard DN, Rich DP, Ostedgaard LS, Gregory RJ, Smith AE, Welsh MJ. Mutations in CFTR associated with mild disease form Cl channels with altered pore properties. *Nature* 1993; 362: 160–164.

4. Welsh MJ, Smith AE. Molecular mechanisms of CFTR chloride channel dysfunction in cystic fibrosis. *Cell* 1993; 73: 1251 – 1254.
5. Kiesewetter S, Macek MJ, Davis C, *et al.* A mutation in CFTR produces different phenotypes depending on chromosomal background. *Nature Genet* 1993; 5: 274 – 278.
6. Chu C-S, Trapnell, Murtagh JJ, *et al.* Variable deletion of exon 9 coding sequences in cystic fibrosis transmembrane conductance regulator gene mRNA transcripts in normal bronchial epithelium. *EMBOJ* 1991; 10: 1355 – 1363.
7. Chu C-S, Trapnell BC, Curristan S, Cutting GR, Crystal RG. Genetic basis of variable exon 9 skipping in cystic fibrosis transmembrane conductance regulator mRNA. *Nature Genet* 1993; 3: 151 – 156.
8. Rosenstein BJ, Cutting GR for the Cystic Fibrosis Foundation Consensus Panel. The diagnosis of cystic fibrosis: a consensus statement. *J Pediatr* 1998; 132: 589 – 595.
9. Dean M, White MB, Amos J, *et al.* Multiple mutations in highly conserved residues are found in mildly affected cystic fibrosis patients. *Cell* 1990; 61: 863 – 870.
10. Miller PW, Hamosh A, Macek M, *et al.* Cystic fibrosis transmembrane conductance regulator (CFTR) gene in allergic bronchopulmonary aspergillosis. *Am J Hum Genet* 1996; 59: 45 – 51.
11. de Braekeleer M, Mari G, Verlingue C, *et al.* Clinical features of cystic fibrosis patients with rare genotypes in Saguenay Lac-Saint-Jean (Quebec, Canada). *Ann Gen* 1997; 40: 205 – 208.
12. Ranieri E, Ryall RG, Morris CP, *et al.* Neonatal screening strategy for cystic fibrosis using immunoreactive trypsinogen and direct gene analysis: four years experience. *BMJ* 1994; 308: 1469 – 1472.
13. Massie RJ, Wilcken B, Van Asperan P, *et al.* Pancreatic function and extended mutation analysis in ΔF508 heterozygous infants with an elevated immunoreactive trypsinogen but normal sweat electrolyte levels. *J Pediatr* 2000; 137: 214 – 220.
14. Kerem B-S, Rommens JM, Buchanan JA, *et al.* Identification of the cystic fibrosis gene: genetic analysis. *Science* 1989; 245: 1073 – 1079.
15. Ferrie RM, Schwarz MJ, Robertson NH, Vaundin S, Super M. Development, multiplexing and application or ARMS tests for common mutations in the CFTR gene. *Am J Hum Genet* 1992; 51: 251 – 262.
16. Chillon M, Casals T, Mercia B, *et al.* Mutations in the cystic fibrosis gene in patients with congenital absence of the vas deferens. *N Engl J Med* 1995; 332: 1475 – 1480.
17. Gibson LE, Cooke RE. A test for concentration of electrolytes in sweat in cystic fibrosis of the pancreas utilising pilocarpine by iontophoresis. *Pediatrics* 1959; 23: 158 – 161.
18. Friedman KJ, Heim RA, Knowles MR, Silverman LM. Rapid characterisation of the variable length polythymidine tract in the cystic fibrosis (CFTR) gene: association of the 5T allele with selected CFTR mutations and its incidence in atypical sinopulmonary disease. *Hum Mutation* 1997; 10: 108 – 115.
19. Cuppens H, Lin W, Jaspers M, *et al.* Polyvariant mutant cystic fibrosis transmembrane conductance regulator genes. The polymorphic (TG)m locus explains the partial penetrance of the T5 polymorphisms as a disease mutation. *J Clin Invest* 1998; 101: 487 – 496.
20. Chmiel JF, Drumm M, Konstan MW, Ferkol TW, Kerscmar CM. Pitfalls in the use of genotype analysis as the sole diagnostic criterion for cystic fibrosis. *Pediatrics* 1999; 103: 823 – 826.