EDITOR'S CHOICE

The very low penetrance of cystic fibrosis for the R117H mutation: a reappraisal for genetic counselling and newborn screening

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ABSTRACT

Background: Cystic fibrosis (CF) is caused by compound heterozygosity or homozygosity of CF transmembrane conductance regulator gene (*CFTR*) mutations. Phenotypic variability associated with certain mutations makes genetic counselling difficult, notably for R117H, whose disease phenotype varies from asymptomatic to classical CF. The high frequency of R117H observed in CF newborn screening has also introduced diagnostic dilemmas. The aim of this study was to evaluate the disease penetrance for R117H in order to improve clinical practice.

Methods: The phenotypes in all individuals identified in France as compound heterozygous for R117H and F508del, the most frequent CF mutation, were described. The allelic prevalences of R117H (p_{R117H}), on either intron 8 T5 or T7 background, and F508del ($p_{F508del}$) were determined in the French population, to permit an evaluation of the penetrance of CF for the [R117H]+[F508del] genotype.

Results: Clinical details were documented for 184 [R117H]+[F508del] individuals, including 72 newborns. The disease phenotype was predominantly mild; one child had classical CF, and three adults' severe pulmonary symptoms. In 5245 healthy adults, $p_{F508del}$ was 1.06%, $p_{R117H;T7}$ 0.27% and $p_{R117H;T5}<0.01\%$. The theoretical number of [R117H;T7]+[F508del] individuals in the French population was estimated at 3650, whereas only 112 were known with CF related symptoms (3.1%). The penetrance of classical CF for [R117H;T7]+[F508del] was estimated at 0.03% and that of severe CF in adulthood at 0.06%.

Conclusions: These results suggest that R117H should be withdrawn from CF mutation panels used for screening programmes. The real impact of so-called disease mutations should be assessed before including them in newborn or preconceptional carrier screening programmes.

Cystic fibrosis (CF, MIM 219700) is one of the most frequent life shortening autosomal recessive diseases, characterised in its classical form by chronic pulmonary obstruction and infections, pancreatic insufficiency, male infertility and elevated sweat chloride concentrations.[1] Over 1500 sequence variations in the *Cystic Fibrosis Transmembrane conductance Regulator* (*CFTR*) gene have been identified in CF and in a number of CFTR-related disorders (CFTR-RD).[2] CFTR-RD are defined as clinical entities where there is evidence of CFTR dysfunction but where the criteria for the diagnosis of CF are not met,[3] and mostly include isolated male infertility due to congenital bilateral absence of the vas deferens (CBAVD (MIM 277180),[4] disseminated bronchiectasis[5 6] and chronic pancreatitis.[7 8] *CFTR* mutations have diverse effects on CFTR protein expression and function.[9] The wide phenotypic spectrum associated with some genotypes, probably influenced by environmental and modifying genetic factors,[3] complicates genetic counselling.

The R117H mutation was initially considered as a mutation causing mild CF with pancreatic sufficiency,[10] in keeping with a residual CFTR function supported by in vitro studies.[11] R117H was then considered as predominantly associated with isolated CBAVD,[4 12–14] generally in compound heterozygosity with F508del, the most frequent CF mutation. Incidentally, the [R117H]+[F508del] genotype was observed in asymptomatic individuals.[15] Phenotypic variability was mostly attributed to the presence of a polypyrimidine variant in the intron 8 acceptor splice site (T5 or T7) in *cis* with R117H, leading to R117H;T5 or R117H;T7 alleles. While the T7 variant is considered neutral, the T5 variant was shown to affect exon 9 splicing reducing the amount of CFTR protein, probably causing a more severe phenotype.[15 16] It was therefore suggested to consider R117H as a CF mutation only in the context of the R117;T5 complex allele.[17] However, early pulmonary manifestations were reported in patients carrying a severe CF mutation and R117H;T7.[16 18–20] The implementation of systematic CF newborn screening (CFNBS) since 2002 in France, relying on determination of immunoreactive trypsinemia (IRT) and subsequent

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screening for 30 common *CFTR* mutations when IRT is above 65 μg/l, led to observe a much higher frequency of R117H;T7 in this population than in CF patients,[21] adding further to the diagnostic dilemma.[16 21–24] To assist with genetic counselling and to improve diagnostic practice, we implemented a large collaborative study to: (1) delineate the overall disease phenotype associated with R117H, and (2) evaluate the penetrance of CF in carriers of the [R117H]+[F508del] genotype—that is, the probability of individuals with this genotype to develop CF. Another issue was to determine to what extent CFNBS methods select for [R117H]+[F508del] newborns.

SUBJECTS AND METHODS

Cross-sectional phenotypic study of individuals compound heterozygous for R117H and F508del

All individuals known to the French CF Laboratory Network before 1 January 2008 who were heterozygous for R117H and F508del were included. These were part of a larger record of all individuals carrying two *CFTR* mutations with R117H at least on one allele (manuscript in preparation). *CFTR* gene analysis had been performed for a CF or CFTR-RD phenotype, a positive family history, or in the framework of CFNBS. Data were centralised at the Centre of Clinical Investigation in Dijon. Genotype data, including the intron 8 poly(T) variant in *cis* of R117H, were collected by the French CF Laboratory Network. Clinical details were provided by the referring physicians on standardised forms including: gender; date of birth; reason for referral (classical CF presentation, respiratory symptoms, chronic sinus disease, pancreatitis, CBAVD, fetal bowel anomalies, positive family history or CFNBS); clinical features together with age at their diagnosis (including pulmonary, gastrointestinal, pancreatic, nasopharyngeal symptoms, CBAVD, foetal bowel abnormalities); neonatal IRT; sputum or oropharyngeal cultures; pulmonary function tests (PFT); sweat chloride concentrations determined by either Gibson and Cooke or Exsudose techniques[25] (indicative of CF: ⩾60 mmol/l; borderline: 30–59 mmol/l in newborns and 40–59 mmol/l in older patients[26]).

Kaplan–Meier analysis was used to estimate the cumulative probability of observing clinical features at different ages. Stata software version 10 was used for statistical analyses.

Epidemiological study in the French general population

The allelic prevalence of R117H (p_{R117H}) and F508del ($p_{F508del}$) and 95% confidence interval (CI) were determined by allele counting in a sample of healthy adult individuals of the French general population. Data were compiled from the French CF Laboratory Network as results of screening for about 30 frequent *CFTR* mutations (including R117H and F508del), performed between 2002 and 2006 in healthy partners of CF carriers or CF patients. These individuals had no personal or family history of CF or CFTR-RD.

Based on p_{R117H} and $p_{F508del}$ and assuming Hardy–Weinberg equilibrium, the number of individuals expected to carry the [R117H]+[F508del] genotype in the French population was estimated to be $p_{[R117H]+[F508del]} = 2 \times p_{R117H} \times p_{F508del}$.

Evaluation of the penetrance of CF in individuals with the [R117H]+[F508del] genotype

The penetrance was defined as the observed number of [R117H]+[F508del] patients with clinical CF, divided by the expected number of individuals with the [R117H]+[F508del] genotype, calculated as defined above.

Epidemiological record in newborns screened for CF

To determine whether elevated IRT selects for [R117H]+[F508del] newborns, the number of [R117H]+[F508del] newborns identified through elevated IRT was compared with the total number of newborns expected to carry this genotype regardless of IRT, based on $2 \times p_{R117H} \times p_{F508del}$. Exhaustive data on newborns screened for CF from the 2002–2006 period, provided by the French Association for the Screening and Prevention of Infant Handicaps (AFDPHE), comprised: (1) the total number of newborns screened (NBS); (2) the number of newborns with elevated IRT; (3) the number of newborns with elevated IRT and the [R117H]+[F508del] genotype; (4) intron 8 poly(T) alleles in these children; (5) the number of newborns with elevated IRT and the F508del homozygous genotype.

RESULTS

Phenotypic description of patients compound heterozygous for R117H and F508del

Among 278 individuals carrying two *CFTR* mutations with R117H at least on one allele, 193 [R117H]+[F508del] individuals were identified: 121 were referred for diagnosis request or positive family history and 72 were discovered through CFNBS (fig 1A). Clinical data were available from 184/193 (95.3%), including 112 individuals who were not referred through newborn screening (non-NBS) and all NBS individuals (fig 1B) (detailed phenotypic description: manuscript in preparation). The poly(T) genotype was known in 173/184 (94.0%) individuals: 166/173 (96.0%) carried T7 and seven (4.0%) T5 in *cis* with R117H. Clinical presentations according to poly(T) variants are shown on table 1.

All individuals were living, apart from one non-NBS patient, who died of chronic hepatitis at 71 years. In the non-NBS group, sweat chloride concentrations using Gibson and Cooke or Exsudose techniques were available in 46 individuals: they were positive (⩾60 mmol/l) in 14 (30.4%), and borderline (30–59 mmol/l) in 18 (39.1%). In the NBS group, 55/72 children (76.4%) were asymptomatic. Sweat chloride values using Gibson and Cooke or Exsudose techniques, available in 56 children, were ⩾60 mmol/l in 4 (7.1%) and from 30–59 mmol/l in 26 (46.4%). No correlation could be found between sweat chloride values and the phenotype—that is, in particular whether non-NBS patients had isolated CBAVD or a multi-organ disease with pulmonary symptoms.

The probabilities of occurrence of different symptoms with age were determined by Kaplan–Meyer analysis for the 166 [R117H;T7]+[F508del] patients (table 2). Pancreatic insufficiency was found in only three patients; in contrast, respiratory symptoms were quite frequent but generally moderate. Adult onset disseminated bronchiectasis was observed in seven non-NBS patients; *Pseudomonas aeruginosa* positive sputum cultures were recorded in only one non-NBS patient but six NBS children, three of whom had a positive sweat test (60, 72 and 76 mmol/l). One child, identified through CFNBS, presented with a classical CF phenotype, including recurrent severe pulmonary obstruction and infections, *P aeruginosa* colonisation and pancreatic insufficiency. She had a positive sweat test and nasal potential difference measurements identical to those found in classical CF. Comprehensive *CFTR* gene analysis failed to identify a further mutation or large gene rearrangement in *cis* with R117H;T7 in this child. The low number of patients with the T5 variant precluded any comparison of the phenotypes associated with R117H;T5 and R117H;T7.

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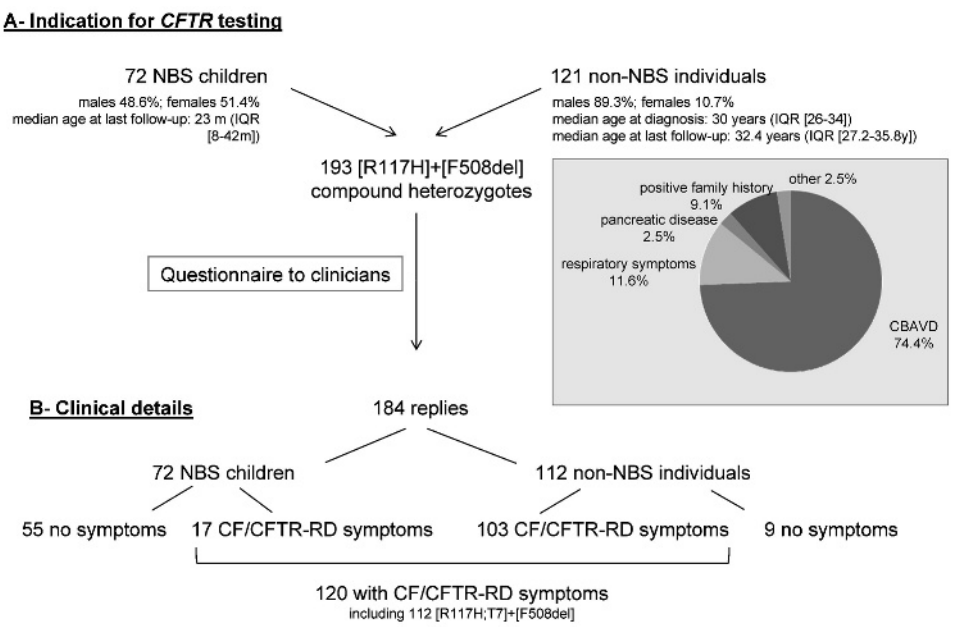

Figure 1 Cross-sectional phenotypic study in [R117H]+[F508del] compound heterozygous individuals. (A) Indications for *CFTR* testing, as reported by the French CF Laboratory Network. Other indications include: hyperechogenic bowel (n = 2) and constipation (n = 1). y: years; m: months. (B) Clinical details made available by the referring clinicians. Newborn screened (NBS) children and non-NBS individuals are indicated separately. In total, 120 patients carrying the [R117H]+[F508del] genotype were recorded with cystic fibrosis (CF) or CFTR related disorders (CFTR-RD) symptoms, of whom 112 had the R117H;T7 allele. The 72 NBS children included the 52 recorded for epidemiological data.

Epidemiological data in the French general population

A total of 5245 healthy adults with no family history of CF (10 490 chromosomes) were screened for frequent *CFTR* mutations. Of these, 151 carried one mutation (observed carrier frequency: 1/34.7), including 111 F508del ($p_{F508del} = 1.06\%$, 95% CI 0.87% to 1.27%) and 30 R117H ($p_{R117H} = 0.29\%$, 95% CI 0.19% to 0.41%) (fig 2A). No compound heterozygote was found. The intron 8 poly(T) variant was determined in 28/30 R117H carriers: all had the T7 variant. $p_{R117H;T7}$ was thus 0.27% (95% CI 0.17% to 0.37%) and $p_{R117H;T5}<0.01\%$. Based on these data, and in the absence of selective mortality associated with the [R117H;T7]+[F508del] genotype, the expected $p_{[R117H;T7]+[F508del]}$ is 1/17 470 ($2\times0.0106\times0.0027$, 95% CI 0.0001% to 0.032%), equivalent to 3650 individuals in the French population of 63 750 000 inhabitants.

Evaluation of the penetrance of CF in individuals with the [R117H;T7]+[F508del] genotype

As R117H;T5 was not detected in the sample of 5245 healthy adult individuals, the penetrance was evaluated only for the [R117H;T7]+[F508del] genotype. While 3650 French individuals would be expected to carry the [R117H;T7]+[F508del]

Table 1 Clinical presentations in the 184 [F508del]+[R117H] compound heterozygous individuals, according to the poly(T) variant

	NBS children (n = 72)		Non-NBS individuals (n = 112)	
	Asymptomatic (No. of individuals)	CF/CFTR-RD symptoms (No. of individuals)	Asymptomatic (No. of individuals)	CF/CFTR-RD symptoms (No. of individuals)
R117H;T7 (n = 166)	47	14: – 1 classical CF – 3 moderate pulmonary + nasopharyngeal – 7 moderate pulmonary – 3 nasopharyngeal	7	98: – 1 severe DB +CRS +CBAVD – 1 severe DB+CRS – 2 DB+CBAVD – 2 DB – 7 moderate pulmonary+CRS+CBAVD – 9 moderate pulmonary+CBAVD – 4 moderate pulmonary+CRS – 1 moderate pulmonary+pancreatic+CBAVD – 7 moderate pulmonary – 6 CRS+CBAVD – 3 pancreatic+CBAVD – 1 CRS – 2 pancreatic – 52 isolated CBAVD
R117H;T5 (n = 7)	2	1: – 1 nasopharyngeal	1	3: – 1 severe DB+CRS – 1 moderate pulmonary – 1 isolated CBAVD
R117H;T?‡ (n = 11)	6	2: – 1 moderate pulmonary – 1 pancreatic	1	2: – 2 isolated CBAVD
Total	55	17	9	103

CBAVD, congenital bilateral absence of vas deferens; CRS, chronic rhinosinusitis; DB, disseminated bronchiectasis; NBS, newborn screened; Non-NBS, non-newborn screened (individuals who were not referred through newborn screening); R117H;T?, cases where the poly(T) variant was not determined or documented.

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Table 2 Probability of occurrence of clinical features at specific age in 166 French [F508del]+[R117H;T7] compound heterozygous individuals

	NBS children (n = 61)			Non-NBS individuals (n = 105)				
	No. of affected cases/no. of patients with available data	Frequency (%)	Penetrance of clinical features (%)* at age (years)	No. of affected cases/no. of patients with available data	Frequency (%)	Penetrance of clinical features (%)* at age (years)		
			2 (99% CI)			2 (99% CI)	10 (99% CI)	30 (99% CI)
Pulmonary symptoms	10/59	17	21 (10 to 42)	29/96	30	8 (3 to 19)	16 (8 to 28)	28 (17 to 42)
Asthma	7/59	12	15 (6 to 37)	9/96	9	4 (1 to 14)	8 (4 to 20)	10 (4 to 21)
Disseminated bronchiectasis	0/59	0	0 (NA)	7/96	7	0 (NA)	0 (NA)	5 (1 to 16)
Nasopharyngeal symptoms	7/59	12	14 (5 to 37)	18/92	20	5 (2 to 16)	9 (4 to 21)	20 (11 to 34)
Chronic sinusitis	7/59	12	14 (5 to 37)	13/92	14	5 (2 to 16)	10 (4 to 22)	15 (8 to 28)
Nasal polyposis	0/59	0	0 (NA)	7/92	8	0 (NA)	0 (NA)	8 (3 to 21)
Pancreatic symptoms	1/59	2	2 (0 to 20)	7/84	8	0 (NA)	0 (NA)	8 (3 to 23)
Pancreatic insufficiency	1/59	2	2 (0 to 20)	2/84	2	0 (NA)	0 (NA)	2 (0 to 19)
Chronic pancreatitis	0/59	0	0 (NA)	0/84	0	0 (NA)	0 (NA)	0 (NA)
Acute pancreatitis	0/59	0	0 (NA)	5/84	6	0 (NA)	0 (NA)	4 (1 to 18)
Staphylococcus aureus positive sputum/oropharyngeal cultures†	19/49	39	37 (21 to 59)	10/37	27	0 (NA)	12 (3 to 36)	31 (13 to 63)
Pseudomonas aeruginosa positive sputum/oropharyngeal cultures†	6/49	12	13 (4 to 35)	1/39	3	0 (NA)	0 (NA)	0 (NA)

NA, not applicable; NBS, newborn screened; non-NBS, individuals who were not referred through newborn screening.
*Results are Kaplan–Meier estimates
†Oropharyngeal cultures in NBS children; sputum cultures in non-NBS individuals.

genotype, only 112 (3.1%) with CF related symptoms were counted in the record of the French CF Laboratory Network, comprising 14 NBS and 98 non-NBS (table 1). Only one case of classical CF in childhood was recorded, which estimates the penetrance of classical CF in childhood, irrespective of age, as 0.03% (1/3650, 95% CI 0.0007% to 0.1526%). Two adult patients had CF with a severe pulmonary disease (disseminated bronchiectasis and forced expiratory volume in 1 s (FEV_1) <60% of predicted value), for an estimated penetrance of delayed severe CF symptoms in adulthood of 0.06% (2/3650, 95% CI 0.0066% to 0.1978%).

Epidemiological data in newborns screened for CF

From 2002 to 2006, 3 527 352 newborns were screened in the CFNBS programmes. Based on the allele frequencies determined in the general population, 202 newborns with [R117H;T7]+[F508del] and 396 with [F508del]+[F508del] were expected among these newborns, irrespective of IRT values.

A total of 23 150 newborns with elevated IRT were identified (0.66%). Of these, 52 were compound heterozygous for R117H and F508del (included in the 72 NBS children of the phenotypic study) and 332 homozygous for F508del. All R117H alleles were found on the intron 8 T7 background (fig 2B). Consequently, an

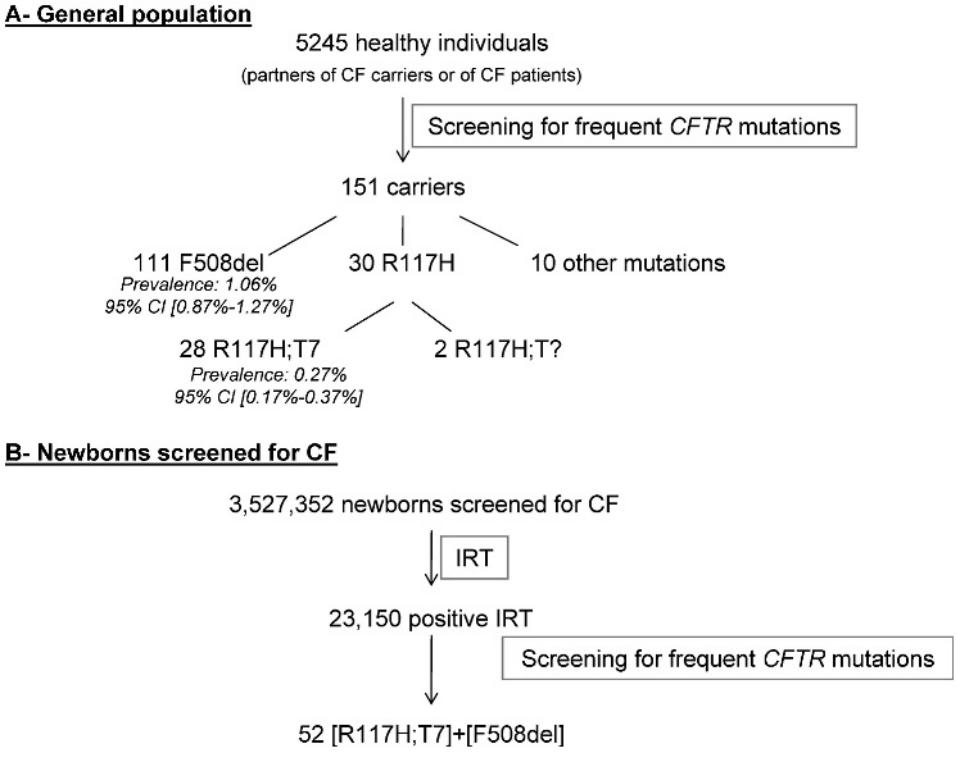

Figure 2 Epidemiological data in (A) the French general population and (B) newborn screened (NBS) children. For clarity, data regarding frequent mutations other than R117H and F508del are not indicated. Data regarding the general population were collected from the French CF Laboratory Network. Those regarding cystic fibrosis (CF) newborn screening for the 2002–2006 period were made available by the French Association for the Screening and Prevention of Infant Handicaps (AFDPHE). R117H;T?: designates cases where the intron 8 poly(T) variant was not determined or documented. IRT, immunoreactive trypsinemia.

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estimated 25.7% (95% CI 19.9% to 32.3%) of newborns carrying the [R117H;T7]+[F508del] genotype were identified through CFNBS, as compared with 83.8% (95% CI 79.8% to 87.3%) of F508del homozygous newborns ($p<0.001$).

DISCUSSION

A very low disease penetrance for R117H

Because of the need for accuracy in genetic counselling, diagnosis and follow-up in newborns carrying R117H and a CF causing mutation, a national collaborative study in France was set up to establish the overall phenotype associated with R117H and to evaluate the disease penetrance of the [R117H]+[F508del] genotype. The measured prevalence of F508del and R117H alleles in the French general population were very similar to those found in a US population ($p_{R117H} = 0.29\%$ *vs* 0.3% and $p_{F508del} = 1.06\%$ *vs* 1.2%).[27] Moreover, according to the overall incidence of CF recently reassessed in France at 1/4136 and F508del frequency at 67.2% of CF chromosomes,[21] $p_{F508del}$ in the general population would be expected to be 1.05%, which is also very close to the value we determined. Therefore, the selection of healthy individuals, partners of CF carriers with no familial history of CF or CFTR-RD did not lead to any evident bias in allele prevalence estimation. The prevalences of F508del and R117H;T7 thus permitted the estimation of the number of individuals expected to carry the [R117H;T7]+[F508del] genotype in the French general population at 3650.

Our study revealed that only 3.1% (112/3650) of the individuals expected to carry the [R117H;T7]+[F508del] genotype have been tested for *CFTR* mutations because of clinical CF or CFTR-RD symptoms. The measured penetrance of severe CF in childhood in this group was only 0.03% and that of delayed severe CF symptoms in adulthood only 0.06%. The penetrance of CFTR-RD cannot be precisely evaluated because, despite an exhaustive record of cases by the French CF Laboratory Network, a number of patients with mild pulmonary symptoms, pancreatitis or CBAVD may not be tested for *CFTR* mutations or may ignore their condition throughout their life. The difference in sex ratio in non-NBS individuals (males/females = 8.3) versus NBS individuals (males/females = 0.95) is compatible with CBAVD being the principal reason for referral in the non-NBS group and supports a very low disease penetrance in women. Beside the single case of classical CF, 13 other NBS children had symptoms that were not specific for CF, mostly bronchial hyperreactivity and nasopharyngeal symptoms. *P aeruginosa* was found in oropharyngeal cultures in six NBS children (12%), of whom three had positive sweat tests, but whether this is predictive of susceptibility to *P aeruginosa* infection or colonisation is unclear. In fact, this figure is surprisingly very similar to that of 13% NBS infants with true CF found with *P aeruginosa* positive oropharyngeal cultures by Armstrong *et al*.[28]

Overall, clinical data in NBS children should be taken with caution, except the CF case, because of the very limited follow-up. Data from prospective regular follow-up of these children should improve the diagnostic classification of this cohort.

In other respects, because of a lack of correlation between sweat chloride values and the phenotype, it would be interesting to determine whether other physiological tests, such as nasal potential difference measurements, could be more accurate, especially in NBS children,[29 30] in order to identify those who are prone to develop lung disease requiring regular clinical assessments and treatment.

Phenotypic variability is not fully explained by the IVS8 poly(T) variation

The very low frequency of R117H;T5 in our population (but not T5 in isolation)[31] has precluded any correlation between phenotype and the poly(T) variation in *cis* with R117H. The presence in our study of a severe CF case associated with R117H;T7 and the previously described overlap between patients carrying R117H;T5 and R117H;T7 show that the commonly cited correlation between phenotype and the poly(T) variant in *cis* with R117H has limitations.[16 18–20 32] However, as R117H;T5 seems to be more frequent in Australian and UK populations and has been associated with a more severe phenotype than R117H;T7,[15 16] determination of the poly(T) variant may still be recommended whenever R117H is detected and R117H;T5 cautiously be considered as a mild CF mutation. Interestingly, five of the seven cases with R117H;T5 originated from Normandy, a north-western area of France which may have common ancestry with the UK.

To what extent does CFNBS detect newborns with R117H?

Based on $p_{R117H;T7}$ and $p_{F508del}$ in the general population, one quarter of [R117H;T7]+[F508del] newborns appear to have elevated IRT and be identified through CFNBS, as compared with 83.7% of expected F508del homozygous newborns. This figure of 83.7%, instead of 100%, could be attributed to the loss of F508del homozygous fetuses due to prenatal diagnoses rather than to incomplete penetrance of the F508del homozygous genotype or overestimation of F508del prevalence, as discussed above. Furthermore, given the low disease penetrance detected in the present study for the [R117H;T7]+[F508del] genotype, we could hypothesise that only a minority of the newborns would develop clinical CF symptoms. These data show that the [R117H;T7]+[F508del] genotype is selected by CFNBS and provide further evidence that not only classical CF but also equivocal cases, CFTR-RD and a number of asymptomatic cases are selected by CFNBS.[21 33]

Implications for CFNBS and genetic counselling

The pertinence of including R117H and mild CF mutations in CFNBS mutation panels has been debated because of the consequences of making a genetic diagnosis of CF in newborns who might not become symptomatic for years, if at all.[16 19 22–24 26 34 35] Bearing in mind that the aim of CFNBS is the earlier diagnosis of classical forms of CF, our results provide strong

Key points

- The penetrance of classical cystic fibrosis (CF) for the [R117H;T7]+[F508del] genotype was evaluated at 0.03%, and that of severe CF in adulthood at 0.06%.
- It was estimated that only 3.1% of individuals expected to carry the [R117H;T7]+[F508del] genotype in the French population have been tested for *CFTR* mutations because of clinical CF or CFTR-RD symptoms or a positive family history.
- R117H on an intron 8 T7 background should be considered principally as a CFTR-RD-associated mutation with reduced penetrance.
- CF newborn screening identifies not only classical CF but also equivocal cases, CFTR-RD and a number of asymptomatic cases.
- Withdrawal of R117H from CF mutation panels used for screening programmes should be considered.

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arguments in favour of removing R117H from CFNBS mutation panels. However, as withdrawal of R117H would lead to further *CFTR* testing in NBS children with abnormal neonatal IRT and a positive or borderline sweat test, inclusion of R117H in a second-step panel would be a good compromise. With regard to diagnosis and genetic counselling, R117H;T7 should be considered principally as a CFTR-RD associated mutation with reduced penetrance. This is of critical importance, as CFNBS is being implemented in an increasing number of countries.[26 36 37] As a result, it does not seem justified to automatically offer prenatal diagnosis to the parents of asymptomatic NBS children and cascade screening to their healthy relatives. Moreover, removal of R117H from CF mutation panels used for carrier screening should be considered, as healthy carriers of R117H could wrongly be considered CF carriers and prenatal diagnosis be improperly performed.

Finally, this study highlights the need to assess the real impact of so-called disease mutations carefully before including them in screening programmes, either for newborn or carrier screening programmes.

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