Genetics inMedicine | ORIGINAL RESEARCH ARTICLE

Comprehensive *CFTR* gene analysis of the French cystic fibrosis screened newborn cohort: implications for diagnosis, genetic counseling, and mutation-specific therapy

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Purpose: Newborn screening (NBS) for cystic fibrosis (CF) was implemented throughout France in 2002. It involves a four-tiered procedure: immunoreactive trypsin (IRT)/DNA/IRT/sweat test was implemented throughout France in 2002. The aim of this study was to assess the performance of molecular *CFTR* gene analysis from the French NBS cohort, to evaluate CF incidence, mutation detection rate, and allelic heterogeneity.

Methods: During the 8-year period, 5,947,148 newborns were screened for cystic fibrosis. The data were collected by the Association Française pour le Dépistage et la Prévention des Handicaps de l'Enfant. The mutations identified were classified into four groups based on their potential for causing disease, and a diagnostic algorithm was proposed.

Results: Combining the genetic and sweat test results, 1,160 neonates were diagnosed as having cystic fibrosis. The corresponding incidence, including both the meconium ileus (MI) and false-negative cases, was calculated at 1 in 4,726 live births. The CF30 kit, completed with a comprehensive *CFTR* gene analysis, provides an excellent detection rate of 99.77% for the mutated alleles, enabling the identification of a complete genotype in 99.55% of affected neonates. With more than 200 different mutations characterized, we confirmed the French allelic heterogeneity.

Conclusion: The very good sensitivity, specificity, and positive predictive value obtained suggest that the four-tiered IRT/DNA/IRT/sweat test procedure may provide an effective strategy for newborn screening for cystic fibrosis.

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Key Words: allelic heterogeneity; cystic fibrosis; immunoreactive trypsin; incidence; newborn screening

INTRODUCTION

Cystic fibrosis (CF; OMIM 219700), a multisystem disease in which lung involvement is the major cause of morbidity and mortality worldwide, remains the most common life-limiting autosomal recessive disease in Caucasian populations. It results from mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*; OMIM 602421) gene and is characterized by exocrine secretion abnormalities, chronic pulmonary deterioration with obstructions and infections, pancreatic insufficiency, hepatobiliary manifestations, male infertility, and elevated sweat chloride concentrations.

More than 1,900 sequence variations (disease and non–disease causing mutations) have been reported in the *CFTR* gene (Cystic Fibrosis Genetic Analysis Consortium, http://www.genet.sickkids.on.ca/CFTR/app). It is difficult to predict the ultimate clinical outcomes of particular *CFTR* mutations because gene modifiers and environmental factors combine in determining the phenotypic severity of lung disease.[1]

Diagnosis of CF, based on clinical symptoms, is often delayed because of the lack of specificity of the wide variety of presenting symptoms. Newborn screening (NBS) therefore has been advocated to reduce delays in diagnosis and facilitate early preventive care with respiratory and nutritional treatment. NBS has been reported to be beneficial, even in the context of modern treatments.[2–6] Based on previous French regional pilot studies[7,8] and international studies, as well as the demonstration that the immunoreactive trypsin (IRT) assay combined with molecular analysis for CF mutations is a feasible method for routine screening,[9] the regulatory agencies (the national health insurance funding agency (Caisse Nationale d'Assurance Maladie des Travailleurs Salariés) and the Ministry of Health) mandated the Association Française pour le Dépistage et la Prévention des Handicaps de l'Enfant to organize systematic CF NBS in France and La Réunion Island under the same framework as other NBS tests. The four-tiered screening protocol involves an IRT/DNA/fail-safe IRT/sweat test (ST) procedure.

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The present study reports the performance of comprehensive molecular *CFTR* gene analysis in the French CF-NBS cohort using data collected during an 8-year period from 1 January 2002 to 31 December 2009 and evaluates the CF incidence, the mutation detection rate, and the allelic heterogeneity in France.

MATERIALS AND METHODS

Patients

France is divided into 22 administrative regions, each of them having its screening laboratory linked to a regional association. The systematic region-by-region national NBS program was implemented from January 2002 to early 2003, and data were collected from 1 January 2002 to 31 December 2009.

Dried blood samples were obtained at 3 days of age for almost all neonates born during the studied period (n = 5,947,148). To fulfill requirements of French bioethical legislation for genetic analysis, written informed consent for DNA testing was obtained from parents and recorded on the back of the filter paper.[10] All data were rendered anonymous, and processing was declared to the Data Protection Commission (Commission Nationale Informatique et Libertés) under the number 1150693.

Screening strategy

During the first month of the program, the protocol flowchart was modified twice.[11] First, because the percentage of neonates with an IRT at day 3 was above the target cutoff of 0.5% (i.e., 0.82%), the day 3 IRT cutoff was raised from 60 to 65 ng/ml. Second, it was decided to repeat IRT at day 21 for infants with a day 3 IRT ≥100 ng/ml and to refer neonates with a day 21 IRT ≥40 ng/ml to a CF center.

The following four-tiered screening strategy was used:

1. IRT measurement at 3 days of age using a radiolabeled immunoassay (RIA-gnost trypsin neonatal kit, CISBIO International, Bagnols/Cèze, France) or an enzyme-linked immunospecific assay (Delfia Neonatal IRT Kit, PerkinElmer, Wallac Oy, Finland) in one of the 22 regional screening laboratories.
2. Investigation of the 30 most common mutations responsible for CF (**Table 1**) using a CF30 Kit (Elucigene CF30, Gen-Probe, San Diego, CA) when the IRT value was above the 99.5th percentile (65 ng/ml). This "French" kit was developed to ensure ≥80% mutation detection in all regions of France, based on a previous national study in symptomatic CF patients,[12] and was validated by a working group mandated by the Association Française pour le Dépistage et la Prévention des Handicaps de l'Enfant.
3. IRT retest at 21 days of age if no mutation was identified in hypertrypsinogenemic neonates with day 3 IRT above the 99.9th percentile (100 ng/ml), or if written consent for DNA testing was not obtained.
4. ST analysis in a CF care center to determine CF or non-CF status when one or two mutations were identified or, in the absence of mutation, when day 21 IRT exceeded 40 ng/ml (99.5th percentile). According to published guidelines,[13–16] the reference ranges of neonate sweat chloride levels are: <30 mmol/l^{-1}, negative; 30–59 mmol/l^{-1}, borderline; and ≥ 60 mmol/l^{-1}, positive.

Genetic laboratories

French molecular genetics laboratories are typically organized into two levels:

1. Level 1: Nine laboratories (located in Brest, Caen, Lille, Lyon, Reims, Montpellier, Paris (Trousseau Hospital and Necker Hospital), and Toulouse) were associated with the NBS program; rapid standardized tests were performed to identify the 30 most common mutations in all neonates with positive day 3 IRT.
2. Level 2: French CF network. In case of hypertrypsinemia with one or no mutations on the CF30 test and a borderline or positive ST, new blood samples underwent complementary analysis in one of the four reference laboratories (Brest, Créteil, Montpellier, Paris-Cochin) or six specialized laboratories (Angers, Bordeaux, Lille, Lyon, Poitiers, Toulouse) of the French CF network. As a second-line diagnostic test for neonates in whom CF or CFTR-related disorders (CFTR-RDs) have already been diagnosed by ST and clinical assessment, these laboratories offer comprehensive *CFTR* gene analysis by scanning methods such as denaturing gradient gel electrophoresis,[17] denaturing high-performance liquid chromatography,[18] or high-resolution melting[19] on all 27 exons and their intron boundaries to identify point mutations or small insertions/deletions, followed by sequencing of polymerase chain reaction products displaying abnormal patterns, and semiquantitative fluorescent multiplex polymerase chain reaction[20] or CFTR Vs03 MLPA assay (MRC-Holland; Amsterdam, The Netherlands) to detect large rearrangements.

Referral to a CF center, ST, and clinical presentation

In parallel to the CF-NBS program, clinical CF centers (Centres de Ressources et de Compétences de la Mucoviscidose [CRCMs]) have been set up for multidisciplinary management and follow-up. The missions of the CRCMs are, among others, to confirm and to explain the diagnosis of CF and to ensure follow-up for all screened CF patients.[11]

The ST, which is the gold standard to confirm or rule out a diagnosis of CF, particularly when mutations of uncertain clinical relevance are identified, was performed at the first CRCM consultation, in line with French guidelines.[21] False-negative results were monitored by the CRCM using an annual questionnaire.

Program surveillance and data collection

The data were centralized by the Association Française pour le Dépistage et la Prévention des Handicaps de l'Enfant. From screening laboratories and regional associations, the monthly distribution of IRT values and the number of molecular biology

Table 1 Allelic frequencies of CF30-kit mutations, identified in neonates with CF, and correspondence between traditional mutation nomenclature and that on the Human Genome Variation Society website

Frequency (F) %	Mutation	Legacy mutation nomenclature	Number of alleles/2,320	% of alleles/2,320	Cumulative %
≥5	p.Phe508del	F508del	1,560	67.24	67.24
	p.Gly542*	G542X	113	3.19	10.51
	p.Asn1303Lys	N1303K	81	1.98	
	c.1585-1G>A	1717-1G>A	48	1.47	
1.00≥F≥4.99	c.2657+5G>A	2789+5G>A	37	1.42	
	p.Arg553*	R553X	36	1.29	
	p.Gly551Asp	G551D	31	1.16	
	p.Tyr122*	Y122X	26	0.97	6.86
	c.2988+1G>A	3120+1G>A	22	0.82	
	c.579+1G>T	711+1G>T	18	0.67	
	p.Ile507del	I507del	17	0.63	
	c.3140-26A>G	3272-26A>G	16	0.59	
0.40≥F≥0.99	p.Arg347Pro	R347P	15	0.56	
	p.Arg1162*	R1162X	15	0.56	
	p.Trp1282*	W1282X	14	0.52	
	p.Tyr1092*	Y1092X	13	0.48	
	c.2051_2052delinsG	2183AA>G	12	0.45	
	c.3528delC	3659delC	11	0.41	
	c.1680-886A>G	1811+1.6kbA>G	9	0.39	
	p.Gly85Glu	G85E	8	0.34	3.06
	p.Ser1251Asn	S1251N	7	0.30	
	p.Arg334Trp	R334W	7	0.30	
	p.Arg117His	R117H	7	0.30	
0.1≥F≥0.39	p.Trp846*	W846X	6	0.26	
	c.489+1G>T	621+1G>T	6	0.26	
	c.948delT	1078delT	5	0.22	
	p.Ala455Glu	A455E	5	0.22	
	p.Glu60*	E60X	4	0.17	
	c.262_263delTT	394delTT	4	0.17	
	c.3718-2477C>T	3849+10kbC>T	3	0.13	
	Total		2,034	87.67	87.67

Mutations are clustered into four groups of frequency intervals (>5%, 1–4.99%, 0.99–0.4%, and <0.4%). The total number of alleles in the 1,160 neonates classified as having CF was 2,320.

CF, cystic fibrosis.

tests were collected. The following items for referred infants were collected from the CF centers: IRT results, genotype, ST values, and initial clinical symptoms. During the period of the present study, data were analyzed every 3 months by the technical committee of the Association Française pour le Dépistage et la Prévention des Handicaps de l'Enfant in order to verify the performance of the kit and to monitor the percentage of positive screens.

Nomenclature

The international nomenclature recommended by the Human Genome Variation Society (http://www.hgvs.org/mutnomen/) was followed. For convenience, the previous legacy mutation nomenclature initially used by the International Consortium Mutation Database (http://www.genet.sickkids.on.ca/CFTR/app), which follows *CFTR* gene numbering (GenBank NM_00492.3 with the A of the ATG translation start codon numbered +133), is also shown in parentheses at the first occurrence in the text.

Classification of mutations and genotypes

In CF, as in many other diseases, the term "mutation" is commonly used to indicate "a disease-causing change," and the term "polymorphism" is used to indicate "a non–disease causing change" or "a change found at a frequency of 1% or higher in the population." To avoid repetition and to prevent any confusion, because the molecular abnormalities described in this article cannot all be classified as "CF-causing" mutations, we use the terms "variations," "variants," or "molecular defects" to designate the different nucleotide changes identified.

Mutations. Because only a limited number of functional studies have assessed the pathogenicity of variants, mutations have been classified in previous studies according to their disease-causing potential.[16,22,23] Based on the recommendations and data from these studies (UMD-CFTR-France),[24] variants were classified into four groups: A, CF-causing; B, associated with CFTR-RDs; C, no clinical consequences; and D, unknown or

uncertain clinical relevance. Some molecular defects associated with a wide phenotypic spectrum might belong to either group A or group B and therefore were classified as group A/B.

Genotypes. According to the classification of the mutations or variants (A, B, C, or D) and the ST values, neonates were classified according to the diagnostic algorithm shown in **Figure 1b**. Neonates carrying a homozygous AA genotype, a compound heterozygous genotype for A and A/B mutation and with positive ST were classified as CF. The CF status of other neonates (AB, AC, AD, A/–) was based on ST values; in cases of positive ST, the children were classified as having CF; if the ST was negative or borderline, they were classified as having CFTR-RDs; and when no ST results were available, neonates remained unclassified.

Statistical analysis

Statistical analysis was conducted using an Excel spreadsheet and EpiInfo software (version 6.04). The incidence with its 95% confidence interval (95% CI) was determined for the whole study period. The validity of the screening protocol was assessed by estimating the sensitivity, specificity, and positive predictive value and negative predictive value parameters. Mutation frequencies were compared using the χ^2 test. All tests were two-sided, and a *P* value less than 5% was considered significant.

RESULTS

Overall data of the NBS program

Dried blood samples were obtained at 3 days of age for almost all neonates (less than 0.01% of parents refused NBS). Between 2002 and 31 December 2009, 34,845 of the 5,947,148 screened newborns (0.58%) had a day 3 IRT test value above the cutoff level and underwent *CFTR* DNA 30-mutation analysis (**Figure 1a**). Of these, 1,005 and 2,830, respectively, had two or one mutation; 16,201 neonates were recalled; and 92.72% (n = 15,023) had a repeat IRT test at day 21. A total of 5,492 neonates were referred to a CF center for ST.

Finally, according to IRT findings, DNA analysis and ST values, or clinical symptoms, the number of neonates screened positive for CF was 1,347.

Three hundred forty-two of these had one or no mutation detected by the CF30 panel, and samples from 341 (one family refused complementary analysis) underwent comprehensive gene analysis in 1 of the 10 specialized laboratories. As a result, 198 other mutations or variations were identified in an additional 335 neonates. After comprehensive gene analysis, only seven infants had one unidentified allele.

Mutations and molecular defects

According to their predicted clinical consequences, the mutations were clustered into four groups. CF-causing mutations (group A) comprised 142 mutations responsible for classic CF. CFTR-related disorder–associated variants (group B) comprised 13 alterations, including one splice variant and 12 missense mutations. p.Arg117His (R117H) was the second most frequent alteration in the cohort as a whole (7.8% of patients; n = 105). The phenotypic variability of this mutation was mostly attributable to the presence of a polypyrimidine variant with seven (T7) or five (T5) thymidines in the intron 9 (IVS8) acceptor splice site, affecting the splicing efficiency of exon 9.[25,26] T5 causes a more severe phenotype, and T7 is considered a neutral mutation. In our population, all individuals carried T7.

Some molecular defects that could belong to either the CF-causing group or the CFTR-related disorders group (group A/B) were reported in patients presenting a broad spectrum of phenotypes from classic CF to mild monosymptomatic presentations.[16] These are four missense mutations (p.Leu206Trp (L206W), p.Arg347His (R347H), p.Asp1152His (D1152H), and p.Ser945Leu (S945L)) and three splice mutations (c.2657+5G>A (2789+5G>A), c.3718-2477C>T (3849+10kbC>T), and c.1210-34TG(13);1210-12T(5) (TG13T5)).

Sixty-four other variants of unknown or uncertain clinical relevance (group D) were identified in 3 homozygous and 81 compound heterozygous patients. For these variants, the pathogenicity could not be predicted *a priori* because these mutations were rare or were reported here for the first time.

Based solely on the CF30 results, at least 1 mutant allele was identified in 1,317 (97.77%) neonates, 1,005 of whom (74.61%) had a complete genotype. A total of 30 neonates had no identified mutation; these and the 312 with one mutant allele underwent comprehensive gene analysis in 1 of the 10 level 2 laboratories, resulting in identification of a complete genotype in 1,340 of the 1,347 (99.48%) neonates.

Classification of neonates and incidence

Combining the results of exhaustive genetic testing and STs, neonates were classified according to the diagnostic algorithm as shown in **Figure 1b**: 1,160 neonates carrying two CF-causing mutations and/or who were ST-positive were diagnosed with CF; 184 with subthreshold STs and carrying mutations associated with CFTR-related disorders or mutations of unknown or uncertain clinical relevance were classified as having CFTR-RDs; 3 carrying variants of unknown or uncertain clinical relevance without available ST values could not be classified.

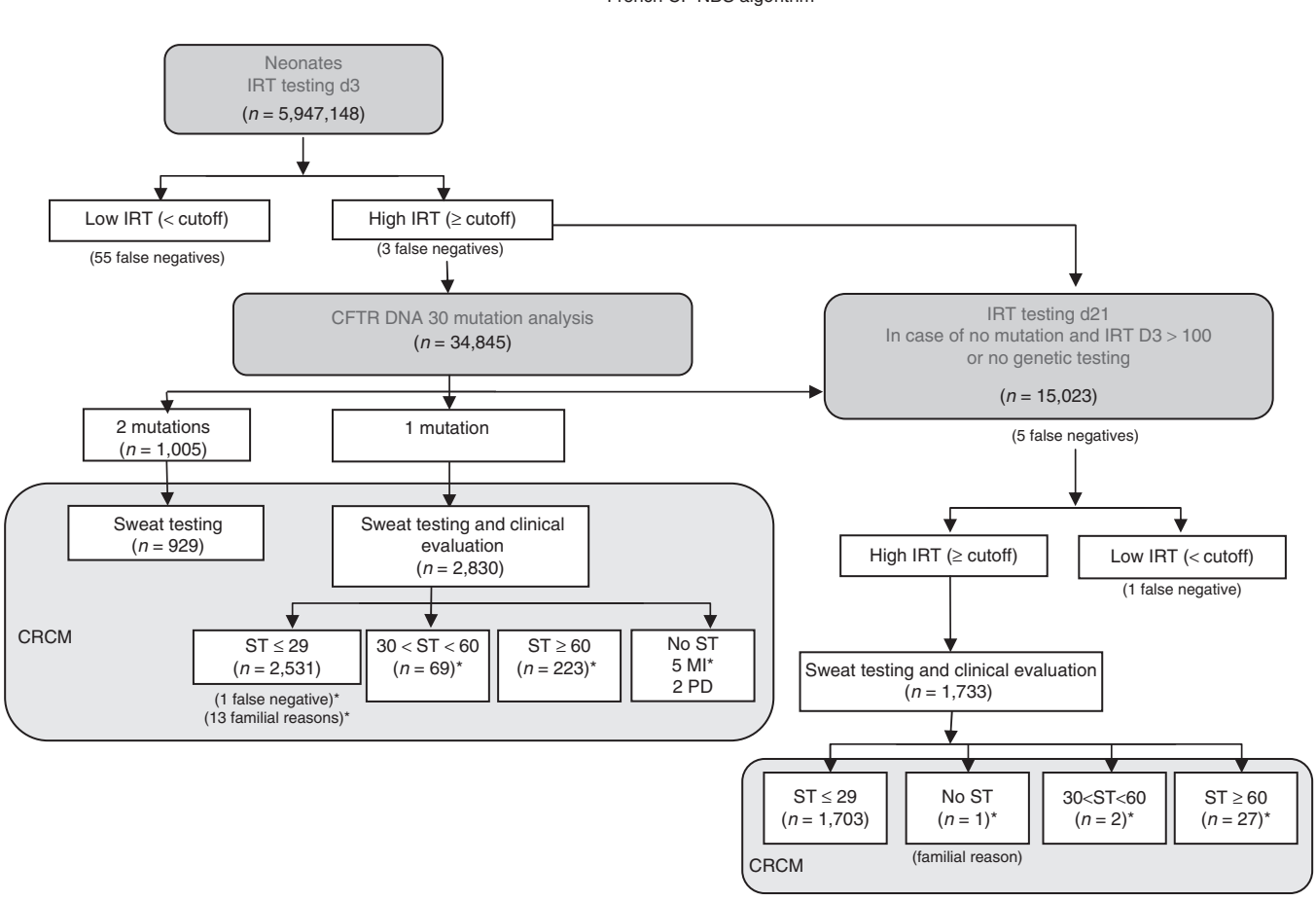
Finally, the 4,234 children with a negative ST and either one *CFTR* gene mutation or no mutation but a persistent hypertrypsinemia at day 21 represent the false-positive results.

The numbers of screened neonates and cases detected, incidence, sensitivity, specificity, and positive and negative predictive values for the screening protocol overall and for CF patients are given in **Table 2**.

The median age at diagnosis for CF children, excluding the MI cases and the neonates born after prenatal diagnosis, was 34 days (range, 6–238; interquartile range, 28–44). It was significantly higher for children who were carriers of one or no mutations of the CF30 kit (43 (interquartile range, 32–61) and 44 (interquartile range, 37–66) days, respectively; $P < 0.0001$).

A total of 577 CF patients (49.74%) were homozygous for mutations identified by the CF30 kit, including p.Phe508del (n = 552, 47.58%), p.Gly542* (n = 5, 0.43%), and p.Asn1303Lys

a **Organizational algorithm**



b **Decision algorithm for classification of the neonates**

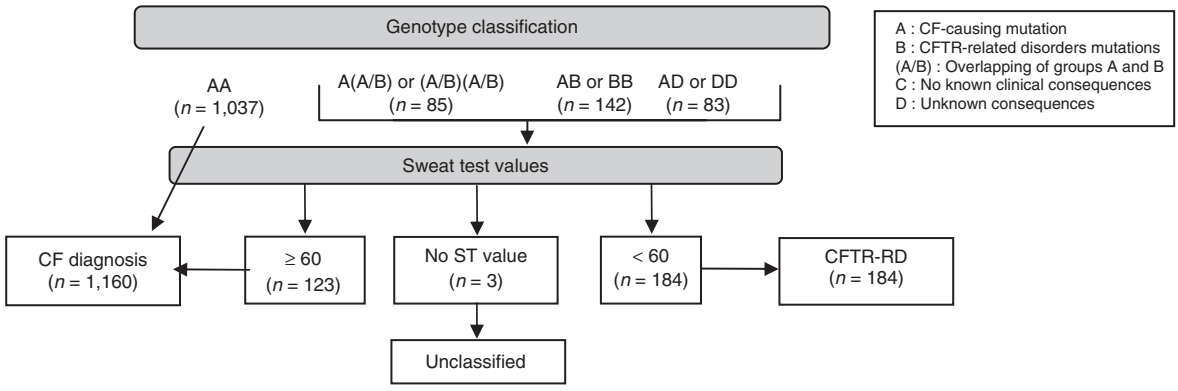


Figure 1 French cystic fibrosis (CF) newborn screening (NBS) algorithm. (**a**) Organizational algorithm. Comprehensive *CFTR* gene analysis is not part of the screening algorithm; it is only performed as a second-line diagnostic test for infants with one or no mutation detected by the CF30 kit (*) in whom CF or CFTR-RD was already diagnosed by sweat test (ST) and clinical assessment. (**b**) Diagnostic algorithm. Neonates carrying a homozygous AA genotype, a compound heterozygous genotype for A and A/B mutations and having a positive sweat test result were classified as having CF. The diagnostic conclusion of the CF status for other neonates (AB, AC, AD, A/–) was based on ST values. In cases of positive ST, the neonates were classified as having CF; if the ST was negative or borderline, then the babies did not meet the CF diagnosis criteria and were classified with an equivocal CF diagnosis or as having CFTR-RD. When the results of ST were not available, the babies remained unclassified. Data shown in this figure concerning the number of newborns tested at day 3, the number of newborns tested at day 21, and the number of CFTR 30 mutation analyses are the real numbers of tests performed by the 22 regional laboratories and the 9 genetic laboratories. The 65 children with false-negative results are divided into (i) 55 children with day 3 immunoreactive trypsin (IRT) levels under the cutoff, (ii) 3 children with day 3 IRT levels above the cutoff but notification failure, (iii) 5 children with day 3 IRT levels less than the day 21 recall cutoff value (i.e., day 3 IRT < 100), (iv) 1 child with a negative sweat test result, and (v) 1 child with day 21 IRT less than the cutoff. CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; CFTR-RD, CFTR-related disorder.

Table 2 Summary of the numbers of newborns screened and cases detected, incidence, and sensitivity, specificity, and predictive values for the screening protocol, for the whole cohort and for the CF patients

	CF	All[a]
Screened newborns	5,947,148	5,947,148
Affected newborns	1,263	1,450
Detected by NBS	1,160	1,347
Missed by NBS	65	65
MI	38	38
CFTR DNA 30-mutation analysis	34,845	34,845
Incidence (95% CI)	2.12/10,000 (2.00/10,000 to 2.24/10,000)	2.26/10,000 (2.14/10,000 to 2.38/10,000))
Sensitivity (%) (95% CI)	95.10 (93.89 to 96.31)	95.40 (94.31 to 96.49)
Sensitivity (%) (95% CI) including MI with low IRT levels as false negative	92.45 (90.99 to 93.90)	92.90 (91.48 to 94.31)
Specificity (%) (95% CI)	99.93 (99.92 to 99.93)	99.93 (99.92 to 99.93)
Positive predictive value (%) (95% CI)	22.26 (21.17 to 23.35)	24.19 (23.07 to 25.31)
Negative predictive value (%) (95% CI)	99.99 (99.98 to 99.99)	99.99 (99.98 to 99.99)

CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; CFTR-RD, CFTR-related disorder; CI, confidence interval; IRT, immunoreactive trypsin; MI, meconium ileus; NBS, newborn screening.

[a]CF, CFTR-RD, and unclassified cases.

(n = 5, 0.43%) (see **Supplementary Table S1** online). In addition 17 were homozygous for a rare mutation.

Those with false-negative results (n = 65) and cases of MI with low IRT levels (n = 38) over the study period were monitored by the questionnaires sent annually to the CF centers (representing 103 children).

Fifty-five of the 65 children with false-negative results had IRT levels at day 3 less than the cutoff level, with a median value of 47 ng/ml (range, 7–64). In six other cases, the children had no mutations detected by the CF30 kit; five of them had IRT levels at day 3 less than the day 21 recall cutoff (median, 88 ng/ml; range, 79–98), whereas the sixth had an IRT at day 21 less than the recall cutoff. There were problems with the notification procedure for the remaining four patients.

Interestingly, cases of MI with low IRT levels (n = 38) represent only 20% of the total cases of MI (n = 196), with median IRT values of 51 and 113 ng/ml for the low and the high IRT group, respectively. Median IRT levels for the infants with MI did not differ from those of the other cases of CF (124 vs. 132 ng/ml; P = 0.31).

Thus, taking infants who met classic CF diagnosis criteria together with both those with false-negative results diagnosed by symptoms and those with MI with IRT levels below cutoff, the incidence of classic CF was 2.12 per 10,000 live births (i.e., 1 in 4,708; 95% CI, 2.00/10,000 to 2.24/10,000). As previously described,[11] we observed a wide range of regional variations ranging from 1 in 3,584 in Lorraine to 1 in 7,309 in Ile de France (**Figure 2**).

Detection rate of the CF30 kit mutation panel and evaluation of allelic heterogeneity in France

The present cohort of more than 1,000 screened neonates with CF presents a unique opportunity to reassess allelic heterogeneity in France and to compare findings with published data.

Table 1 summarizes the frequencies of the mutations identified by the kit in 2,320 alleles. p.Phe508del was present in 1,560 (67.24%) alleles, varying from 50% in Central and Southern France (Regions Auvergne, Franche-Comté, and Provence-Alpes-Côte d'Azur) to 76% in Northern France (Regions Nord-Pas de Calais and Champagne-Ardennes). The variations in p.Phe508del frequency did not correlate with those observed in incidence (r^2 = 0.036). Six other mutations had a relative frequency ≥1%, accounting for a cumulative rate of 10.51%. Another group of 11 mutations had respective frequencies ranging from 0.99 to 0.40%. Moreover, 12 mutations ranging from 0.10 to 0.39% were observed in three to nine alleles.

The CF30 kit identified 87.67% of the 2,320 CF alleles (i.e., 2,034) for a detection rate of ≥80% for all 22 French regions. Two regions with slightly lower detection rates were located in Central and Southern France, i.e., Franche Comté at 78.57% and Provence-Alpes-Côte d'Azur at 76.67% (data not shown).

The mutation spectrum in neonates with CF diagnosed through NBS is consistent with the previously reported spectrum in patients with CF diagnosed based on clinical symptoms.[12] The percentage of the main mutations was similar (**Figure 3**); however, there were significantly higher rates of specific mutations, including p.Tyr122* (Y122X) in CF patients from the Réunion Island (prevalence, 0.97 vs. 0.16%; $P < 10^{-6}$) and c.2988+1G>A (3120+1G>A) (prevalence, 0.82 vs. 0.09%; $P < 10^{-6}$) in patients of African origin.

Furthermore, large rearrangements were identified in 20 of the 2,320 CF alleles (0.86 vs. 0.06%), representing nearly 7% of the alleles not identified through the kit.

After comprehensive scanning, 198 other mutations were identified, with a nearly exhaustive representation of the mutated alleles (99.7%), and only 7 alleles (0.3%) remained unidentified.

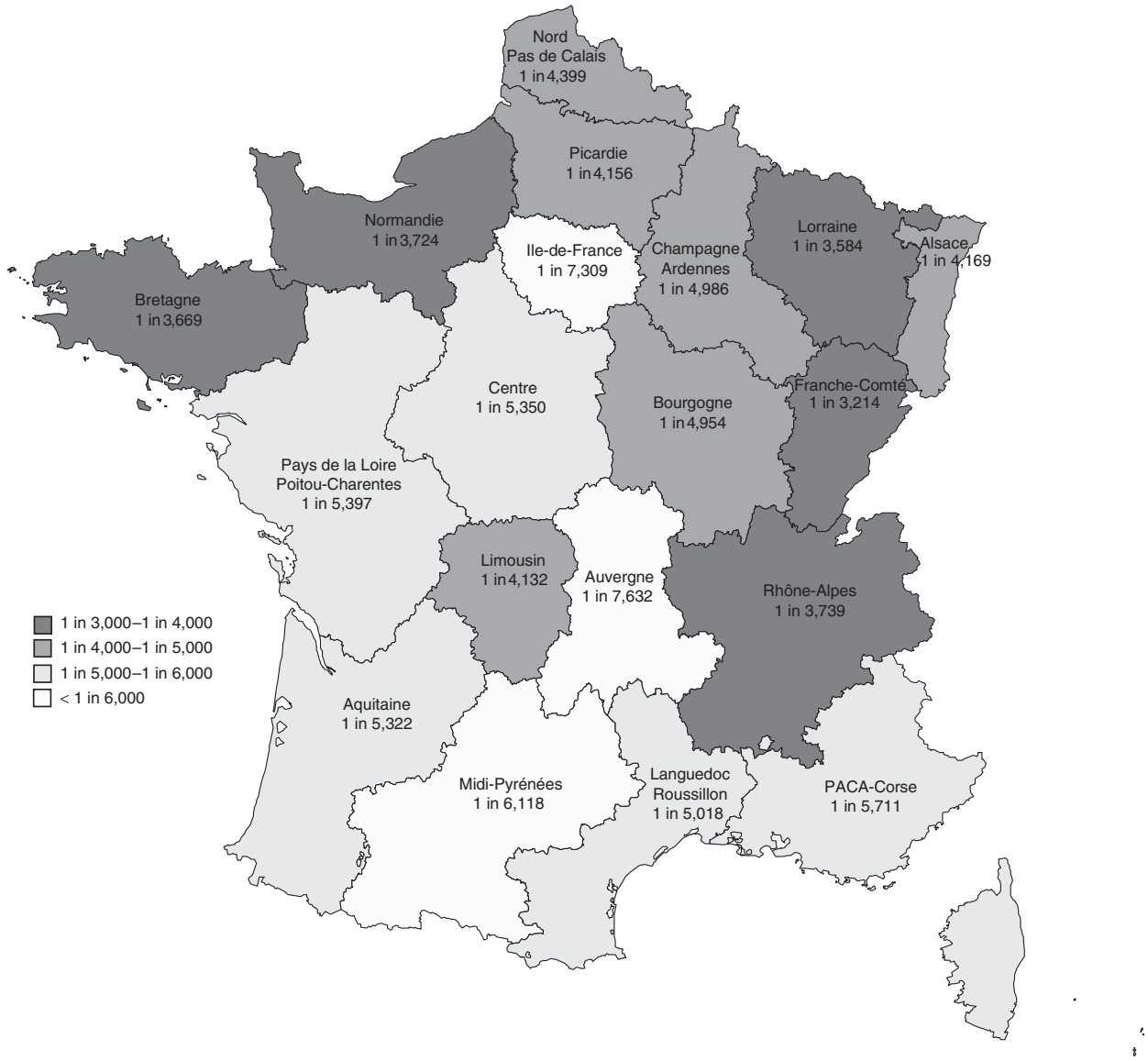


Figure 2 Regional differences in the incidence of cystic fibrosis during the studied period.

DISCUSSION

We report in this study the results of 8 years of the French CF NBS program based on an IRT/DNA protocol, followed by a comprehensive gene analysis in CF children.

Before the implementation of the French CF NBS program, expert groups compiled recommendations for screening strategy, including all technical aspects and the importance of close collaboration between laboratories and clinical CF centers to ensure efficiency. The study confirmed the performance of the CF30 panel and further comprehensive gene analysis, with detection rates of 87.67 and 99.7%, respectively. To our knowledge, this is the highest mutation detection rate reported from a large heterogeneous nationwide population of screened newborns.

After combining genetic and ST results, 1,160 neonates were diagnosed with CF during the study period. A further 38 neonates with MI and IRT levels below cutoff were also considered to have CF, and another cohort of 65 infants with symptoms suggestive of the disease, positive ST, and 2 CF-causing mutations undetected on the NBS program (false-negative results) were reported. Thus, overall, during this period, 1,263 infants were diagnosed with CF, giving a global incidence of 2.12 per 10,000 live births in France (i.e., 1 in 4,708; 95% CI, 2.00/10,000 to 2.24/10,000), which was lower than the previously estimated incidence of CF in a Caucasian population (4.00/10,000 or 1 in 2,500).[27] According to these data, CF allele frequency is estimated at 0.014, corresponding to an expected carrier frequency of 1 in 34.

Further comprehensive gene analysis is sometimes routine, as in the California CF NBS algorithm,[28] but in France it is implemented later in level 2 molecular biology laboratories for a limited number of neonates with positive or borderline STs. It identified 198 other mutations in 99.7% of the alleles, with

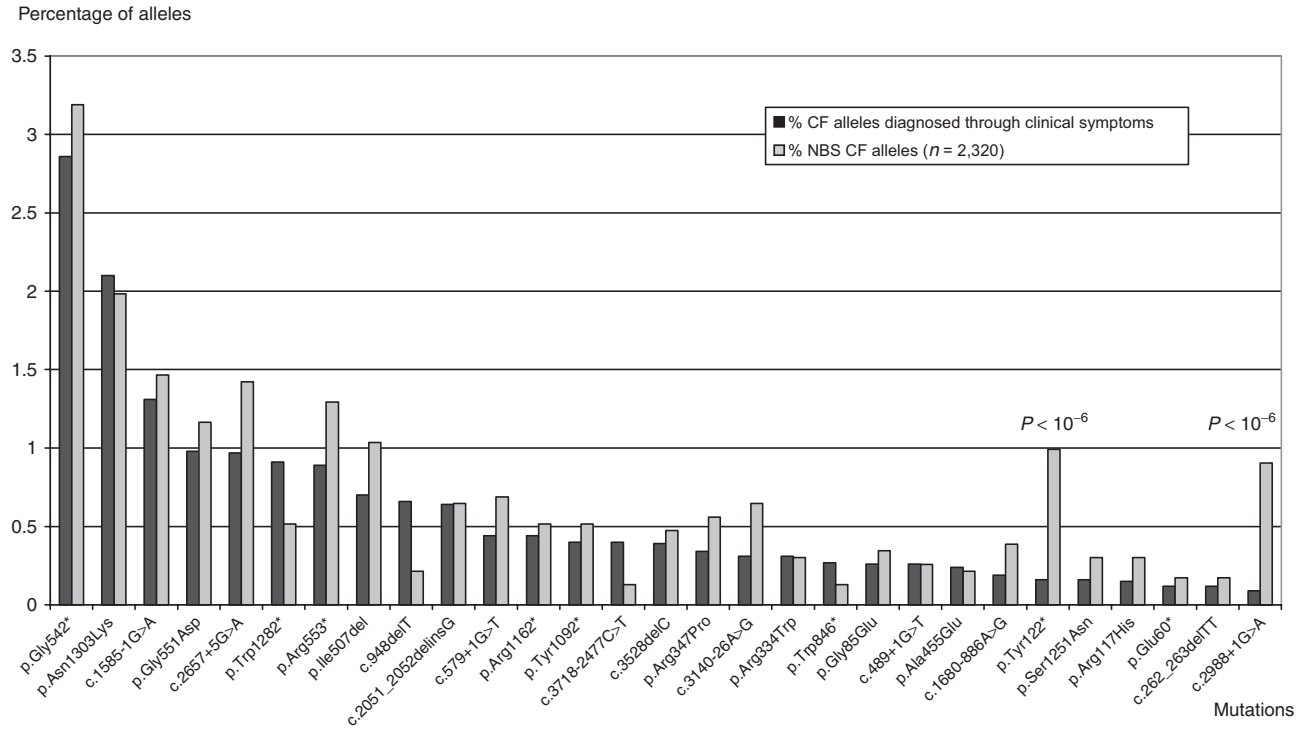


Figure 3 Comparison of the mutation spectrum in French cystic fibrosis (CF) patients for the mutations included in the CF30 kit, except for the p.Phe508del mutation. Dark gray, patients diagnosed based on clinical symptoms; light gray, patients identified based on newborn screening. Statistical analysis found a significant difference between patients diagnosed based on clinical symptoms and those based on newborn screening for the frequencies of the c.2988+1G>A and p.Tyr122* mutations. NBS, newborn screening.

only 7 unidentified alleles (including one case in which the parents refused complementary analysis), reflecting the allelic heterogeneity found in France and in other countries, such as the Czech Republic.[29]

Sensitivity and specificity were greater than 95% and 99%, respectively, equivalent to the findings of the Australian study,[30] which was the closest to the present study in terms of strategy and number of neonates screened. The present positive predictive value was better, possibly because of the panel of mutations analyzed.

The current nationwide French NBS algorithm for CF, which combines IRT assay/DNA analysis, fail-safe IRT, and STs, provided a good detection rate for infants with classic CF, with only 5.6% false-negative results. We cannot be sure we have detected all the false-negative cases, but clinical symptoms of CF appear early in life and we think that after 3 years, the large majority of these cases have been diagnosed.

One point of concern, however, is that NBS identifies not only classic CF but also a small proportion of neonates with CFTR-RDs, i.e., infants with borderline STs and one or two detected variations, and infants with STs ≤ 60 mmol/l with two detected mutations on CF30, who do not meet the criteria for the diagnosis of CF. Whether these cases, representing up to 13.66% of our overall cohort, half of which carry a p.Arg117His mutation, might benefit from NBS is highly questionable. Recently, Thauvin et al.[31] assessed individuals carrying a p.Arg117His mutation and a CF-causing mutation, showing classic CF penetrance of 0.03% and severe CF penetrance in adulthood of 0.06%. Considering that the aim of NBS is early diagnosis of classic forms of CF, their findings provide a strong argument for removing the p.Arg117His mutation from the *CFTR* NBS mutation panel.

Clearly, genetic information concerning *CFTR* mutations is increasingly important, not only for more accurate genetic counseling of families but also for the development of new therapeutic approaches targeting specific mutations. Mutations can have different impacts on the CFTR protein, from the absence of synthesis to malfunction. Depending on their functional impact, new therapeutic approaches have emerged, with mutation-specific treatments that target specific gene defects.[32] Potentially active agents have been identified and analyzed, resulting in several new compounds and, in one case (VX-770 for the p.Gly551Asp mutation),[33] in a license in the United States and Europe. Treatments will clearly be mutation-based, and these data suggest that CFTR-targeted drugs might arrest disease progression and perhaps hamper the development of CF disease in infants diagnosed by NBS. Thus, the patient's genotype is the cornerstone of this approach, facilitating selection of patients to whom clinical trials can be offered.

Conclusion

In this study, we reported the results of a nationwide CF NBS program based on an IRT/DNA protocol, followed by a comprehensive *CFTR* gene analysis in children with CF. To our

knowledge, the present detection rate is the highest reported from a large heterogeneous nationwide population of screened newborns.

Based on these results of the French 8-year experience with nearly 6 million newborns, we show that the 4-tiered IRT/DNA/IRT/ST CF-NBS procedure is an effective strategy for NBS for CF that could be easily implemented in other countries.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/gim

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DISCLOSURE

The authors declare no conflict of interest.

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